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INHERITANCE OF APHID RESISTANCE IN PI 567541B AND PI 567598B, IDENTIFICATION OF APHID RESISTANCE QTL IN PI 567598B, AND A NEW APHID BIOTYPE IN MICHIGAN

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Clarice Mensah

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By

Clarice Mensah

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ABSTRACT

INHERITANCE OF APHID RESISTANCE IN PI 567541B AND PI 567598B, IDENTIFICATION OF APHID RESISTANCE QTL IN PI 567598B, AND A NEW APHID BIOTYPE IN MICHIGAN

By

Clarice Mensah

The soybean aphid (*Aphis glycines* Matsumura) has become a very important pest of soybean [*Glycine max* (L.) Merr.] in North America since it was first reported in 2000. In 2005, four new plant introductions (PI) with aphid resistance: PI 567543C, PI 567597C, PI 567541B and PI 567598B were identified. Since then, other sources of aphid resistance have been identified, but only in two sources has genetic and molecular characterization been conducted. The objectives of this research were to:1) determine the inheritance of antibiosis resistance in PI 567541B and PI 567598B, 2) determine if a different soybean aphid biotype exist in Michigan and 3) identify and map quantitative trait loci (QTL) underlying aphid resistance in PI 567598B. Field studies were conducted to determine the inheritance of antibiosis resistance in PI 567541B and PI 567598B. The two resistant PIs were crossed with one or two susceptible soybean lines and the F_1 and F_2 plants and $F_{2:3}$ families were evaluated for aphid resistance. All F_1 plants were found to be susceptible to soybean aphids. The plants in seven F_2 populations segregated in a 15 susceptible to 1 resistant ratio, which is the expected ratio for a trait controlled by two recessive genes. The segregation data shows that two recessive genes are involved in the

resistance in PI 567541B and PI 567598B. This information will be useful for breeders to design efficient breeding schemes for developing soybean cultivars with resistance to aphids. To achieve our second objective, 188 F_2 individuals from a cross between Titan and PI 567598B were genotyped with 109 polymorphic simple sequence repeats (SSR) markers. Both single marker analysis (SMA) and composite interval mapping (CIM) methods were used to determine locations of QTLs. SMA revealed 24 markers associated with aphid resistance. QTL mapping by CIM identified a putative QTL on LG J. The SSR markers flanking these resistance genes can be used in marker-assisted selection for aphid resistance in soybean breeding programs. With the testing of several soybean aphid resistant genotypes, it was expected that resistant biotypes would evolve. In a field study in 2006, Dowling, a resistant check was found to be susceptible to the soybean aphid. A greenhouse study was conducted to compare the effect of the aphids which overcame the resistance in Dowling and aphids collected in the field in 2006. Dowling was found to be susceptible to both aphid colonies. In a follow up greenhouse study Dowling was found to be resistant to aphid colonies which had been raised in a growth chamber and greenhouse since 2002.These two studies indicate that, there is a difference in the feeding behavior on Dowling by aphids collected in 2002 and 2006 and suggests that a new soybean biotype may have evolved in Michigan.

Dedicated to the three Adovor's in my life: Doe, Volta and Qwekqem

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CHAPTER 1

INTRODUCTION

Soybean

Soybean, *Glycine max* (L.) Merr., (2n=2x=40) is a legume in the Fabaceae family. The genus *Glycine* is divided into two questionably distinct subgenera: *Glycine* and *Soja*. The first consists of six or seven perennial species primarily from Australia. The second consists of three annual species from Asia: *Glycine max*, *Glycine soja*, and *Glycine gracilis* (Palmer et al., 1996). Soybean combines in one crop both the dominant world supply of edible vegetable oil, and the dominant supply of high-protein feed supplements for livestock (Rao, 2002). Other fractions and derivatives of the seed have substantial economic importance in a wide range of industrial, food, pharmaceutical, and agricultural products (Johnson, 1987). As a source of protein, soybean is often less expensive compared to animal protein on a cost per kilogram basis (Hymowitz and Newell 1981).

The worlds leading producer of soybeans is the U.S. followed by Brazil and Argentina (FAO, 2007). In 2006, these three countries produced 82% of the 236 million tonnes of soybean produced worldwide. In Michigan, soybean is the number two crop, in terms of acreage, 1.75 million acres was planted with in 2007 (Soy Stats 2007).

The Soybean Aphid

The soybean aphid (*Aphis glycines* Matsumura) belongs to the order Hemiptera and family Aphididae. It is a small, light yellow or yellowish green aphid with two

distinct black cornicles and a pale colored tail projection. Adult soybean aphids are about 1/16th of an inch (2 mm) long and may be winged or wingless. Immature aphids look like a miniature version of the wingless adults and winged ones have a black head and thorax. Soybean aphids have quickly established themselves as one of the most damaging pests of soybean (Sun et al., 2000). Originally from Asia, aphids were first detected in 2000 in the upper Midwest of the US; they have since spread to several states and some Canadian provinces (Chen et al. 2000). The soybean aphid is the only aphid in North America that develops large colonies on soybeans.

Soybean aphids display a complex life cycle with alternation of sexual and asexual generations and host plants (Ragsdale et al. 2004). In North America, various buckthorn (*Rhamnus carthartica* L.,*Rhamnus alnifolia* L' Hertier) species are used as primary hosts (Voegtlin et al., 2004). Soybeans are the secondary hosts of soybean aphids. The observed life history of the aphid in North America is similar to that observed in China and Japan, with the exception of the primary hosts, *Rhamnus davurica* Pallus and *Rhamnus japonica* Maxim which it uses as an over-wintering host (Takahashi et al., 1993). In spring the wingless mothers hatch from an egg and begin to produce colonies of wingless females, these then produce a third generation of aphids that are winged emigrants which fly in search of soybean, the summer host. All asexual generations are entirely female and are clones of the mother. Winged females occur in the fall as the temperature decreases and plant conditions deteriorate. They then migrate to the buckthorn where they produce wingless females. At this time, winged males occur in the soybean field and migrate to the buckthorn where they mate with the wingless females, which lay over-wintering eggs. The life cycle repeats the next spring.

Numbers of aphid generations range from 10 to 22 per year (Li et al. 2000). In China,Wu et al. (2004) recorded a total of 18 generations per year with 15 of those generations occurring on soybean. Wingless and winged female aphids produce an average of 58 and 38 nymphs, respectively, at 26°C (Li et al. 2000). Winged aphids play a vital role in expanding the range of dispersal within and among fields and migration between alternative host plants. Crowding of wingless adults and poor host quality induce winged aphid production (Lu and Chen 1993).

Symptoms and Damage on Soybeans caused by the Soybean Aphid

Plant damage occurs when large numbers of aphids remove significant amounts of water and nutrients as they feed on leaves and stems, causing leaves to wilt, curl, yellow, and even drop (Wu et al., 2004). Other symptoms include plant stunting, poor pod fill, reduced pod and seed counts, smaller seed sizes, and nutrient deficiencies, resulting in overall yield and quality reduction (DiFonzo and Hines, 2002). Significant yield loss (8-25%) occurs when aphid densities peak at flower initiation. Honeydew, a sticky substance excreted by soybean aphids onto the leaves leads to the development of sooty mold, which affects photosynthesis and results in yield and seed quality loss (Chen and Yu, 1988).

During the feeding process, soybean aphids are capable of transmitting viruses including soybean mosaic, alfalfa mosaic, mungbean mosaic, peanut mosaic, and bean yellow mosaic virus (CAB International, 2001). These viruses commonly occur together and form a disease complex which leads to plant stunting, leaf distortion and mottling, reduced pod numbers and seed discoloration (Glogoza, 2002). Soybean Mosaic Virus

(SMV) is transmitted in a nonpersistent manner and causes high yield loss. It is spread mainly by infected aphids feeding on healthy plants. Epidemics of SMV are dependent not only on the initial virus source but also on the abundance and development of aphid vectors, especially winged aphids. Occurrence of winged aphids in soybean fields has been found to be closely associated with the incidence of SMV (Quimio and Calilung, 1993).

Host Plant Resistance Modalities

Resistant varieties of crop plants have played an important part in controlling many insect, mite and nematode pests. Fewer applications of insecticides are usually needed to control insect pests when resistant varieties are used (Hoffmann et al., 1993). The importance of developing crop plants that are resistant to major insect pest has created the need for examination of the mechanism involved in resistance. The widely recognized classification proposed by Painter (1951), provides an acceptable illustration of the possible basis of resistance. The three mechanisms that influence the ability of a plant to grow productively in the presence of an insect are nonpreference, antibiosis, and tolerance (Painter, 1951). One or more of these mechanisms can be in operation in a plant considered as insect resistant.

Nonpreference refers to behavioral responses of insects to a plant. Kogan and Ortman (1978) suggested the term antixenosis to describe the plant properties responsible for this response. The plant characters that influence nonpreference include color, light reflection, type of pubescence, leaf angle, odor and taste. For example; the yellow-green cultivars of peas are less desirable to the pea aphid than are the blue-green ones. The

cabbage aphid is attracted most to plants with leaves that reflect low intensities of light (Fehr, 1999). Antibiosis refers to plant characteristics and is considered to be the only true form of resistance. It is the type of resistance in which a host plant has a detrimental effect on the physiology and life history of an insect pest. These adverse effects may include inhibited growth, death, and prolonged time to maturity (Painter, 1951).

According to Smith (1989), if a plant deters feeding by an insect, the mechanism of resistance may be either antixenosis or antibiosis. The critical question that separates these two types of resistance is whether the insect is completely prevented from feeding, thus starving to death (antibiosis) or would eventually feed on that plant when given no choice (antixenosis). Often there is an overlap between the antibiosis and antixenosis types of resistance. Complex types of resistance and different combinations of resistance are expected to give more complete and durable control than one simple type. For example, a resistant variety that expresses antibiosis and tolerance to an insect will give excellent and durable control. On the other hand, such a combination might be difficult to select for and manage in a breeding program.

Soybean Aphid Resistance in North America

At the time the aphid arrived in 2000, no known sources of resistance were available in soybean. In 2004, the first aphid resistance sources were reported. After screening 1,542 soybean accessions, Dowling and Jackson, two late maturity ancestral cultivars were found to have antibiosis resistance to the aphids (Hill et. al., 2004). The next report of resistance was from our breeding program here at MSU in 2005. After

evaluating 2,147 soybean germplasm in choice tests we identified four accessions, PI 567598B, PI 567541B, PI 567543C, and PI 567597C, with resistance to the soybean aphid (Mensah, et al., 2005). A no-choice test revealed that PI 567598B and PI 567541B possessed antibiosis resistance (Mensah et al., 2005). In 2006, Diaz-Montana et al. compared the reproduction of soybean aphids on 240 soybean entries and found eleven entries with fewer nymphs than the susceptible checks. In a follow-up experiment, they identified K1639 and Pioneer 95B97 as showing a strong antibiosis effect on soybean aphids. Two more new sources of aphid resistance are PI 230977 and G93-9223 (PI 595099) with antibiosis and antixenosis resistance, respectively (Hesler et al., 2007).The latest report of aphid resistance is of, three PIs (243540, 567301B and 567324) identified by Mian et al., (2008) after screening nearly 200 soybean genotypes in a greenhouse no-choice test. PI 243540 was found to possess antibiosis resistance and PI 567301B and PI 567324 possessed mainly antixenosis resistance.

Inheritance of Aphid Resistance

Resistance to insects is governed by genetic mechanisms like other plant traits (Auclair, 1989). Understanding the inheritance of resistance is necessary for breeders to develop an effective strategy for utilization of resistant germplasm in their breeding programs (Mornhinweg et al., 2002). Knowledge of the inheritance of insect resistance, as with other economic plant traits, helps to design appropriate breeding procedures to develop resistant cultivars. It is also useful for the identification of biotypes of insects that may already exist or develop over time (Smith, 1989). Qualitative, or simply inherited, traits require different breeding methods than quantitative traits controlled by many genes.

In many crops, the next logical step after the discovery of resistance is to determine the mode of inheritance. There are many examples where this has been conducted using a classical genetics approach. In spring barley (*Hordeum vulgare* L.) line STARS-9577B, segregation data of F_2 and BC_1F_1 populations suggested that Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko), resistance is controlled by two dominant alleles (Mornhinweg et al., 2002). Inheritance of resistance to aphid (*Aphis craccivora* Koch) in cowpea (*Vigna unguiculata* (L.) was found to be controlled by a single dominant gene after analyzing data from parental, F_1, F_2, F_3, and backcross populations (Bata et al., 1987). Inheritance of resistance to a wheat midge, *Sitodiplosis mosellana* (Géhin), was investigated in spring wheat derived from nine resistant winter wheat cultivars and was found to be conferred by a single partly dominant gene (Mckenzie et al., 2002). Resistance to the green peach aphid in the red leaf peach rootstock cultivar `Rubira' was found to be controlled by a single dominant gene (Pascal et al., 2002). Resistance to the soybean aphid in the cultivars Dowling and Jackson has been found to be controlled by single dominant genes (Hill et al., 2006a, 2006b).

Biotypes of Insects

The resistance in many cultivars has been effective for only a short period of time due to the emergence of new genotypes of the pest (Fehr, 1999). The protective properties of insect resistant cultivars may be overcome by the development of resistance in insect populations that possess an inherent genetic capability to over come plant resistance (Smith, 1989). Typically, insect biotypes occur in nature as products of a

survival mechanism for the persistence of an insect species and develop as a result of selection of parental populations in response to exposure to resistant cultivars (Smith, 1994). The failure to recognize the existence of biotypes may lead to severe infestations of formerly resistant plants. The study of insect biotypes is a significant part of insect resistance programs as it provides tools for the analysis of insect plant relationships that serve as the basis of breeding resistant plants (Saxena and Barrion, 1987). Identifying insect biotypes can be a long and difficult process. In many insects, biotypes may be determined by the response of a group of differential host varieties to an insect population (Smith, 1994).

Insect Resistance QTL

Linkage drag caused by co-integration of undesirable agronomic traits linked to alleles associated with resistance QTL is a major obstacle to soybean breeders, developing agronomically competitive cultivars with effective insect resistance (Boerma and Walker, 2005). Genetic studies using classical techniques have identified >250 soybean loci since the discovery of the T locus for pubescence color by Piper and Morse's in 1910. In comparison, over 300 QTLs associated with various traits have been identified in soybean using molecular markers since 1990 (Orf et al., 2004). Yencho et al. (2000) listed 233 insect resistance QTLs that have been mapped in six different crop species. Although DNA marker technology is powerful, it nevertheless has limitations in detecting QTLs with relatively small effects (i.e., 'modifier genes'). Of the soybean QTLs reported in the literature, at least 162 appear to condition >10% of the variation in

phenotype, and only a small fraction of the total have actually been confirmed. DNA markers linked to important genes or QTLs can be used for MAS, thereby reducing the need for phenotype-based selection. Tagging these genes with markers also makes it possible to study them in different genetic backgrounds.

Resistance to aphids may be quantitative rather than qualitative in expression. For instance, expression of resistance to the cabbage aphid, *Brevicoryne brassicae* (L.), in the wild species *Brassica fruticulosa* Cirillo is quantitative (Pink et al., 2003). A quantitative trait locus involved in adult plant cereal aphid resistance has also been detected and mapped in barley (Moharramipour et al., 1997). Resistance to other insects in soybeans is quantitative in expression and inheritance (Kilen and Lambert, 1998), including resistance to the Mexican bean beetle, *Epilachna varivestis* (Multsant) (Rufener et al., 1989), and resistance to the corn earworm, *Helicoverpa zea* Boddie, (Rector et al., 2000).

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CHAPTER 2

INHERITANCE OF SOYBEAN APHID RESISTANCE IN PI 567541B AND PI567598B

ABSTRACT

In a previous study, two soybean [*Glycine max* (L.) Merr.] plant introductions (PIs), PI 567541B and PI 567598B, were found to possess antibiosis-type resistance to the soybean aphid (*Aphis glycines* Matsumura). Plants with antibiosis resistance negatively interfere with the reproduction of the aphid and thus control the insect. Field studies were conducted to determine the inheritance of antibiosis resistance in PI 567541B and PI 567598B. The two resistant PIs were crossed with susceptible soybean lines and the F_1 and F_2 plants and $F_{2:3}$ families were evaluated for aphid resistance. All F_1 plants were found to be susceptible to soybean aphids. The plants in seven F_2 populations segregated in a 15 susceptible to 1 resistant ratio, which is the expected ratio for a trait controlled by two recessive genes. The $F_{2:3}$ families also segregated in a 15 susceptible to 1 resistant ratio. Therefore, the segregation data suggest that two major recessive genes are involved in the resistance in PI 567541B and PI 567598B. This information can be used by breeders to design efficient breeding schemes for developing soybean cultivars with antibiosis resistance to aphids.

INTRODUCTION

The soybean aphid (SBA), *Aphis glycines* Matsumura, was first discovered in eight Midwestern U.S. states in 2000. Since then it has spread throughout the north central United States and parts of Canada (NCSRP, 2004) and has become one of the major pests affecting soybean production in North America. SBA populations can double very quickly (McCornack et al., 2004), and aphid numbers can reach thousands per plant. Aphid feeding reduces photosynthesis (Macedo et al., 2003) and reduces yield components including plant height, number of nodes and pods per plant, seed size, and bean quality (DiFonzo and Hines, 2002; Ostlie, 2003). In efficacy trials conducted in Michigan during SBA outbreak years, yield in untreated plots was 18% to 40% less than yield in treated plots (DiFonzo, 2006; Difonzo and Hines, 2002).

Insecticides are still the primary means of controlling SBA, which increase production costs and human exposure to pesticides. In 2005, an outbreak year for SBA across the Midwest, millions of acres were treated (NASS, 2006). Insecticide applications also kill natural enemies of soybean aphids (Smith and Krischik, 1999) and may increase populations of other soybean pests such as spider mites. Host-plant resistance is the most effective means to control insect pests. Soybeans resistant to SBA colonization would eliminate or minimize the need for insecticides, reducing cost, environmental impacts, and exposure.

Since the discovery of SBA in the US, significant effort has been made to identify sources of resistance in soybean. Hill et al. (2004) screened 1,542 soybean accessions and identified seven, including Dowling and Jackson, with resistance to SBA. We evaluated 2,147 soybean germplasm accessions in choice tests and identified four PIs, PI 567598B,

PI 567541B, PI 567543C, and PI 567597C, with resistance to SBA (Mensah et al., 2005). In a no-choice test, PI 567598B and PI 567541B were found to possess antibiosis resistance (Mensah et al., 2005). In 2006, Diaz-Montana et al. compared the reproduction of SBA on 240 soybean entries and identified eleven entries with fewer nymphs than the susceptible checks. In a follow-up experiment they identified K1639 and Pioneer 95B97 as showing a strong antibiosis effect on SBA. Recently, Hesler et al. (2007) have also found two aphid resistance sources, PI 230977 with antibiosis resistance and G93-9223 (PI 595099) with antixenosis resistance. Currently only the resistance in Dowling and Jackson has been characterized; it was shown to be controlled by a single dominant gene (Hill et al., 2006a, 2006b). The inheritance of the other sources of aphid resistance has not yet been characterized.

Development of SBA-resistant cultivars is an objective in many public and private soybean breeding programs in North America. For resistance sources to be useful in developing resistant plants, the genes conferring resistance must be characterized. The number of genes controlling resistance as well as the nature of the resistance determines the breeding method required to transfer this resistance into elite cultivars. The objective of this current study is to determine the inheritance of SBA resistance in the two soybean accessions PI 567598B and PI 567541B that exhibit antibiosis resistance.

MATERIALS AND METHODS

PI 567541B was crossed with E00075 and PI 567598B was crossed with Titan and E00075. Both Titan and E00075 were susceptible to soybean aphids. Each F_1 plant was harvested separately to develop F_2 populations. The parental lines and F_1 plants of the cross Titan x PI 567598B were evaluated for SBA resistance in 2004 and the F_2 populations from the same cross were evaluated in the field during 2005. Parental lines, F_1 plants, and F_2 populations from the crosses E00075 x PI 567541B and E00075 x PI 567598B were evaluated for aphid resistance in the field in 2005. The number of plants in each F_2 population is shown in Table 2.1. Evaluation of SBA resistance was carried out in a 12.2- x 18.3-m aphid-proof cage in the field on the Michigan State University campus in East Lansing, MI. Two weeks after planting, when the plants were at the V2 stage (Fehr and Caviness, 1977), each plant was inoculated with two aphids according to the method described by Mensah et al. (2005). All aphids used in these tests were obtained from nearby naturally infested soybean fields. The F_1 plants were planted 30.5 cm apart with no replication and the parents were planted 5.1 cm apart with two replications. Each F_1, F_2, and parental plant was rated for aphid damage two, three, and four weeks after inoculation using a rating scale of 0 to 4 described by Mensah et al. (2005).

Seeds from 376 individual F_2 plants in population 030104-8 (Table 2.1), which was developed from a single F_1 plant of the Titan x PI 567598B cross, were harvested individually during fall of 2005. The 376 $F_{2:3}$ lines and the parents were evaluated for aphid resistance in the field during summer 2006. Depending on seed availability, up to

fifteen F_3 progenies from each F_2 plant were planted. Resistance evaluations were conducted in a field cage as described previously, but using a modified version of the rating scale described by Mensah et al. (2005). The rating scale used for F_1 and F_2 plants did not clearly distinguish between plants with low (one or two) versus moderate (tens of aphids) infestation. In 2006 half steps were added to the original 0 to 4 scale (Table 5.1, appendix). Over 3000 F_3 plant were rated weekly for three consecutive weeks starting three weeks after inoculation.

When the susceptible parents first rated a score of 4.0, the data from that sample date were used to classify the F_2 or F_3 plants as resistant or susceptible. A plant with a rating of 1.5 or less was classified as resistant, while a plant with a rating larger than 1.5 was considered susceptible. The threshold of 1.5 was comparable to the threshold used to identify susceptible plants in our previous study (Mensah at al., 2005). Chi-square tests were performed to test the goodness of fit of observed segregation ratios among F_2 plants and $F_{2:3}$ families with different genetic ratios, with rejection at 0.05 level of probability.

RESULTS AND DISCUSSION

All F_1 plants from two of the three crosses were found to be susceptible with a rating greater than 1.5 (Table 2.2), suggesting that resistance to SBA is controlled by recessive genes. Data for the F_1 plants in the third cross (Titan x PI 567598B) were not obtained due to poor infection in 2004 as a result of heavy rain and flooding damage after aphid inoculation. The overall frequency distribution of aphid colonization ratings in all F_2 populations was not normal, and was skewed toward the susceptible parents (Figure

2.1), suggesting that susceptibility was dominant over resistance. The distributions were continuous, indicating that more than one gene was involved in aphid resistance in the two PIs and the dominance of susceptibility over resistance was not complete. All the F_2 populations segregated in a 15:1 susceptible/resistant ratio (Table 2.1), which is the expected ratio for a trait controlled by two recessive genes with duplicate dominant epistasis. In both cases when E00075 was crossed with PI 567598B and PI 567541B the resulting F_2 populations also fitted the 15:1 susceptible/resistant ratio confirming the recessive nature of the resistance genes in a different population.

For the Titan x PI 567598B $F_{2:3}$ families, on average, eight seeds per family germinated. Out of the 376 $F_{2:3}$ families 25 were found to be resistant, fitting the 15:1 ratio with a P value of 0.258. Forty five out of 351 $F_{2:3}$ families derived from susceptible F_2 plants segregated for resistance. The recessive nature of the resistance in PI 567598B and PI 567541B was confirmed in the $F_{2:3}$ families as all 25 resistant F_2 individuals produced resistant $F_{2:3}$ families. Due to the recessive nature of resistance in PI 567598B, it was expected that susceptible heterozygotes would segregate when the F_3 families were tested for aphid resistance.

However, segregation was observed only in 45 out of $F_{2:3}$ families. This low number of $F_{2:3}$ segregating families might be due to low seed yield from susceptible F_2 plants and poor germination. Based on Fehr (1987), at least 11 plants are needed to have a 95% chance of identifying one resistant plant with a 0.25 expected frequency. On average, we had only eight plants per family; therefore many families did not have the minimum number of plants required to find a resistant plant in a segregating $F_{2:3}$ family.

The segregation data in the F_2 populations and $F_{2:3}$ families shows that two major recessive genes are involved in aphid resistance in both PI 567598B and PI 567541B. However, from the results there is a possibility that other minor genes are also involved in the resistance.

Insect resistance like all other traits can be controlled by either one or more dominant or recessive genes. SBA resistance in the soybean cultivars Dowling and Jackson is controlled by a single dominant gene (Hill et al., 2006a, 2006b). Our study demonstrated that aphid resistance in the soybean PI 567598B and PI 567541B is controlled by two recessive genes, suggesting different resistant genes from those in Dowling and Jackson underlie the resistance in these two PIs. Little is known about the mechanism of pest resistance in soybean, the involvement of a recessive allele in the antibiosis might be a clue (Komatsu et al. 2005).

Different genes and inheritance patterns for aphid resistance have also been reported in other crops. In wheat, nine characterized genes (Dn1, Dn2, *dn3*, and Dn 4 to Dn9) are involved in resistance to the Russian wheat aphid, *Diuraphis noxia* (Du Toit, 1989; Harvey and Martin, 1990; Liu et al., 2001; Marais and Du Toit, 1993; Marais et al., 1998; Nkongolo et al., 1991a, 1991b; Schroeder-Teeter et al., 1994). Eight of the genes are independent dominant genes each conferring resistance in a different resistance source, while *dn3* is a recessive gene conferring the aphid resistance in *Triticum tauschii.* In barley, a single dominant gene controls the Russian wheat aphid resistance in the line S13 (Robinson et al., 1992) and two dominant genes control resistance in the line STARS-9577B (Mornhinweg et al., 2002).

As with all host plant resistance to insects or pathogens, there is the concern that the resistance will be overcome. In wheat, the resistance gene *Dn4,* found in many varieties, was overcome by a new biotype of Russian wheat aphid found in Colorado in 2003 (Haley et al., 2004). In a follow-up experiment, Haley et al. (2004) found that only one of the nine resistance genes, *Dn7*, conferred resistance to the new biotype. In 2006, three new aphid biotypes were identified based on the foliar damage they caused; one biotype was virulent to eight of the nine sources of Russian wheat aphid resistance in wheat (Burd et al., 2006). Each of the eight sources carried different genes conferring resistance to Russian wheat aphid. The adaptive ability of aphids in general to overcome plant resistance through biotype differentiation highlights the need to explore the genetic diversity of SBA resistance. Variation of SBA biotypes has been observed in the US (Kim et al., 2007; Mensah et al., 2007). Some biotypes have overcome the resistance from Dowling and Jackson but not the resistance from PI 567598B and PI 567541B (Kim et al., 2007; Mensah et al., 2007). Therefore, different sources of resistance must be used to develop SBA resistant cultivars.

In general, resistance controlled by multiple genes is more durable than the resistance controlled by a single dominant gene (Duvick, 1999). Thus the resistance from PI 567541B and PI 567598B may be more durable than the single gene controlled resistance from Dowling and Jackson. However, more effort will be required to incorporate the resistance from these two PIs into elite germplasm because larger progeny populations are required to recover at least one resistant progeny with the resistance.

The information on the recessive inheritance of the SBA resistance detected in this study is useful to breeders in developing special schemes in breeding programs in

order to incorporate this resistance in elite breeding lines. In breeding for insect resistance, backcrossing is the major approach for introducing resistance into an otherwise superior cultivar. Selfing after each backcross can be used to select lines with the recessive resistance gene. If markers associated with the genes are identified, marker-assisted selection can be used to identify resistant lines faster, and therefore incorporation of the recessive genes into new cultivars will be easier and faster (Chen and Line, 1999). Genetic populations have been developed to test for allelism of genes controlling aphid resistance in these two PIs. Research is ongoing to identify molecular markers associated with the resistance genes in this study.

Table 2.1: Segregation of aphid resistance in F_2 populations derived from susceptible x resistant crosses

Population ID†	Susceptible parent	Resistant parent	Total no. of plants	Observed		Expected by a 15:1 (R:S) ratio		P value of X^2 test§
				R‡	S‡	R	S	
040129-1	E00075	PI 567541B	155	5	150	10	145	0.120
040129-2	E00075	PI 567541B	98	5	93	6	92	0.639
040130-1	E00075	PI 567598B	100	7	93	6	94	0.757
040130-2	E00075	PI 567598B	126	8	118	8	118	0.963
030104-3	Titan	PI 567598B	415	26	389	26	389	0.990
030104-8	Titan	PI 567598B	388	32	356	25.	363	0.148
030104-10	Titan	PI 567598B	416	26	390	26	390	1.000

†Each F_2 population was developed from a single F_1 plant. F_2 populations developed from different F_1 plants of the same cross were considered different populations.

‡R = Resistant, S = Susceptible

§If the P value is larger than 0.05, the null hypothesis that the observed R:S ratio fits the expected 1:15 ratio is not rejected statistically.

Table 2.2: F_1 and parental lines classified as resistant to soybean aphid.

Genotype	Total no. of plants tested	No. of resistant plants	Mean rating
PI 567541B	9	9	1.0
PI 567598B	12	12	1.0
E00075	8	0	4.0
Titan	13	0	4.0
(E00075 x PI567541B) F_1	6	0	3.3
(E00075 x PI567598B) F_1	12	0	3.0
(Titan x PI567598B) F_1	10	-	-

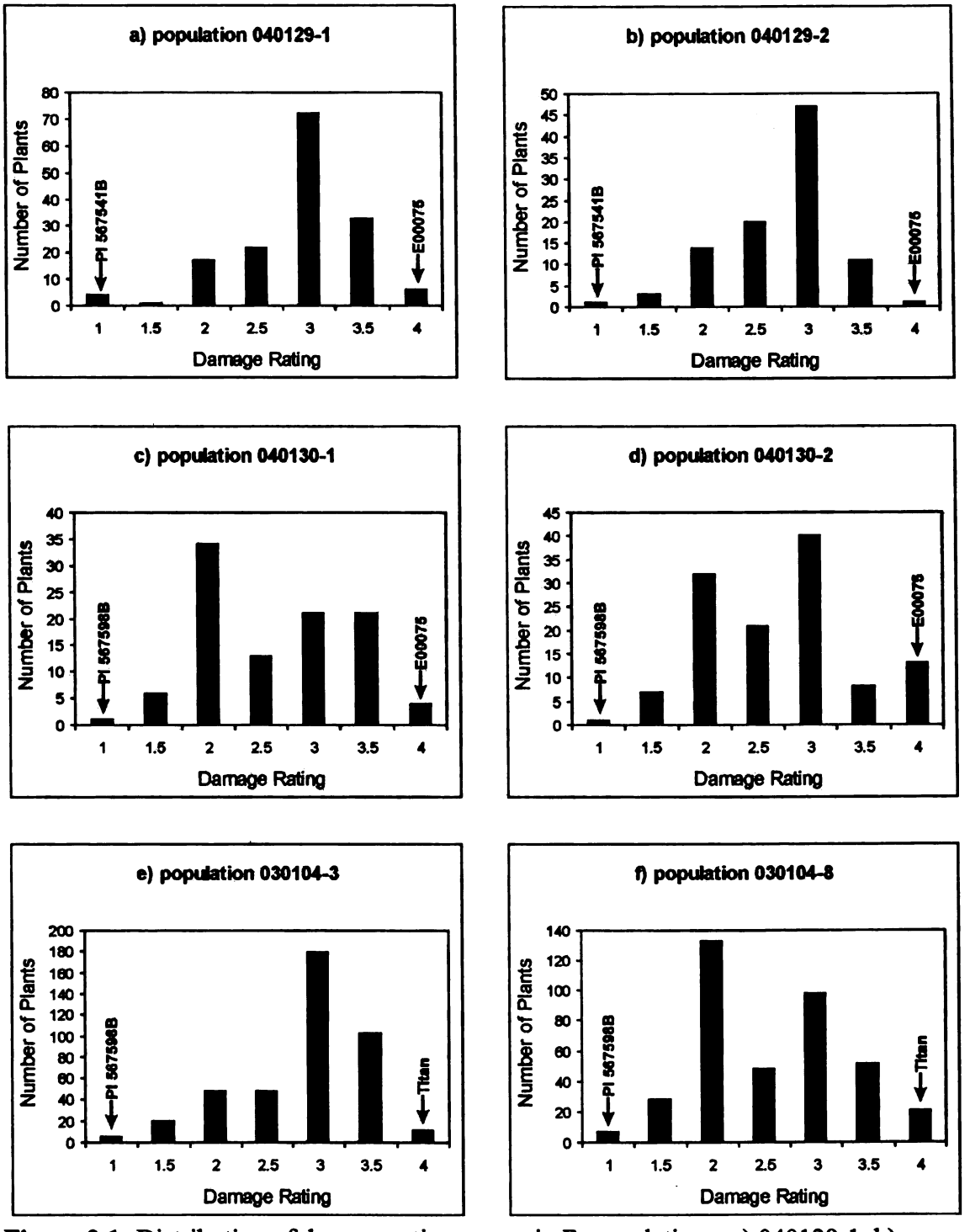

Figure 2.1: Distribution of damage rating scores in F_2 populations: a) 040129-1, b) 040129-2, c) 040130-1, d) 040130-2, e) 030104-3, and f) 030104-8.

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CHAPTER 3

VARIATION IN SOYBEAN APHIDS IN 2006: A CASE FOR THE PRESENCE OF SOYBEAN APHID BIOTYPE DIVERSITY IN MICHIGAN

ABSTRACT

The soybean aphid, *Aphis glycines* (Matsumura), has over the past five years become one of the most important pests of soybean *Glycine max* L. in Michigan. When this research was initiated in 2006, there was no documentation of the existence of biotypes of soybean aphids in North America. However in other aphid species, like the green bug and Russian wheat aphid, biotypes have arisen after the release of aphid resistant crop genotypes. With the testing of several soybean aphid resistant genotypes it is only a matter of time that a new biotype would evolve. In a field study in 2006, Dowling, a resistant check was found to be susceptible to the soybean aphid. The objective of this study was to determine if a new aphid biotype is present in Michigan. A greenhouse study was conducted to compare the effect of the aphids which overcame the resistance in Dowling with random aphids collected in the field in 2006. Dowling was found to be susceptible to both aphid colonies collected in 2006. These results were compared to data from a greenhouse study conducted using aphid colonies that were collected in 2002 and maintained in a growth chamber and greenhouse since 2002. Dowling was found to be resistant to the aphid colony collected in 2002. These results indicate that, there is a difference in the virulence reaction on Dowling by aphids collected in 2002 and 2006. This suggests that a new soybean biotype may have evolved in Michigan.

INTRODUCTION

The soybean aphid has become one of the major economic pests affecting soybean production in North America (Schmidt et al., 2007). In 2005, soybean aphid outbreaks were reported in several states, with millions of acres treated with pesticides in Minnesota, Indiana, and Michigan (O'Neal, 2006). The soybean aphid is the only aphid in North America that develops large colonies on soybean (Plant Health Initiative, 2004). Plant damage occurs when large numbers of aphids remove significant amounts of water and nutrients as they feed on leaves and stems, causing leaves to wilt, curl, yellow, and even drop. Other symptoms of direct feeding damage include plant stunting, poor pod fill, reduced pod and seed counts, smaller seed size, and nutrient deficiencies resulting in overall yield and quality reduction (DiFonzo and Hines, 2002). Significant yield loss (8–25%) occurs when the soybean plants are heavily infested by the aphid during the early reproductive stage (DiFonzo and Hines, 2002; Hunt and Jarvi, 2005).

After a six year observation period, the soybean aphid appears to be on a 2-yr cycle, alternating years with significant economic problems with years where populations are very low or almost non existent (Ragsdale, 2006). The first response to aphid control was the use of insecticides, but these pesticides also killed natural enemies of soybean aphids (Smith and Krischik, 1999). Millions of dollars were spent annually spraying chemicals to control the aphids in infested soybean fields (Li et al., 2007). Host-plant resistance however, is the most effective means of controlling insects as it helps eliminate or minimize the need for insecticides.

Many soybean breeding programs in North America are working to identify soybean genotypes with resistance to the soybean aphid. In 2004, Hill et al., reported

seven accessions, including Dowling (Maturity Group (MG) VIII) and Jackson (MG VII), with antibiosis resistance to the aphids after screening 1,542 soybean accessions. In 2005, the breeding program here at Michigan State University (MSU), identified four (MG III) plant introductions (PIs) PI 567598B, PI 567541B, PI 567543C, and PI 567597C, with resistance to the soybean aphid, after evaluating 2,147 soybean germplasm in choice tests (Mensah, et al., 2005). Diaz-Montana et al. (2006) compared the reproduction of soybean aphids on 240 soybean entries and found eleven entries with fewer nymphs than the susceptible checks. Hesler et al. (2007) have also found two aphid resistance sources, PI 230977 with antibiosis resistance and G93-9223 (PI 595099) with antixenosis resistance. Recently, Mian et al, (2008) found three MG IV PIs (243540, 567301B and 567324) to be resistant to soybean aphids and identified six others which were moderately resistant after screening nearly 200 soybean genotypes (cultivars, breeding lines and PIs) in a greenhouse choice test.

The aphid resistance in each of the two soybean cultivars Dowling and Jackson is controlled by single dominant genes (Hill et al., 2006a, 2006b) *Rag 1* and *Rag* respectively (Hill et al., 2006a, Li et al., 2007). The aphid resistance in the germplasm identified by Mensah et al., (2005) is controlled by two recessive genes. With deployment of resistance sources there is always the concern that biotypes of the insects would arise and overcome resistance. When resistance is controlled by a single dominant gene it is not uncommon for resistance to breakdown in a relatively short time. In other aphid species, the use of cultivars with a single aphid resistance gene has favored the selection and rapid spread of aphid biotypes adapted to this resistance. For example, biotypes of both Russian wheat aphid [*Diuraphis noxia* (Mordvilko)] and greenbug [*Schizaphis*

graminum (Rondani)] were found capable of overcoming resistance genes in new cultivars (Burd and Porter, 2006; Haley et al., 2004). In breeding red raspberry for resistance to the large raspberry aphid (*Amphorophora idaei*) using single major genes or polygenic minor genes proved successful in controlling this virus vector aphid for more than 30 years. Current surveys found that more than 75% of the *A. idaei* populations in the United Kingdom consisted of biotypes with the ability to break the most widely deployed resistance gene, A_{10} (Birch et al., 2006).

In other aphid species similar methods have been used to determine the presence of biotypes. In a Russian wheat aphid study, the performance of two *D. noxia* sources was compared on three wheat cultivars, Trego, Halt, and Stanton, and after characterizing substantial differences in plant responses, it was determined that biotypes were present (Jyoti and Michaud, 2005). In another study, Qureshi et al., (2005) evaluated colonization of commercial wheat cultivars by the two biotypes and reported some differential responses and confirmed the presence of biotypes in Russian wheat aphid.

In the summer of 2006, Dowling (Hill et al., 2004) an aphid resistant cultivar which has been used as a resistant check since 2002 in our breeding program was found to be susceptible to aphids collected in natural occurring colonies in the field. Since the arrival of the soybean aphid there has been no documentation of the presence of biotypes, but this observation led to our current hypothesis that biotypes of soybean aphids may have arisen. The discovery of soybean aphid biotype diversity in Illinois and Ohio has been reported recently (Kim et al., 2008). The objective of this current study is to determine if soybean aphid biotypes have evolved in Michigan. The reaction of aphids

collected in 2006 and 2002 would be compared on selected resistant and susceptible soybean genotypes.

MATERIALS AND METHODS

To determine the possible presence of soybean aphid biotypes in Michigan two studies were conducted. The first study compared the reaction of different resistance sources to aphids collected from a susceptible Dowling plant with aphids collected in the field in 2006. In the second study, selected resistant and susceptible genotypes were infected with aphids that have been kept in a growth chamber and greenhouse since 2002 to verify if the cultivar Dowling was still resistant. This information would help determine genetic differences exist between aphids collected in 2002 from those collected in 2006.

All studies were carried out in the Plant Science Greenhouse, MSU with temperatures between 22 and 25°C. All soybean seeds were planted in a plastic pot 22 cm in diameter and 23 cm deep, the soil used in all cases was Baccto High Porosity Professional Planting mix (Michigan Peat Company, Houston, TX). All plants were inoculated at the V2 stage (Fehr and Caviness, 1977) with two wingless aphids each on the partially expanded trifoliate with a camel-hair brush.

Aphid Culture

In study 1, two sources of aphids were used, Colony A consisted of aphids collected from susceptible Dowling plant in the field in 2006 and raised on Dowling

plants in the greenhouse. Colony B was made up of aphids collected randomly from a seed cage in the field in 2006. The aphids were raised on Williams 82 (susceptible check) in the greenhouse. In study 2 we used aphids which have been maintained in isolation in a growth chamber and greenhouse since 2002. The colony was obtained from the Field Crops Entomology Laboratory, MSU.

Plant materials

In the first study to determine the difference between field collected aphids and aphids from the susceptible Dowling plant, the soybean genotypes used were: PI 567598B, PI 567541B, PI 567543C, and PI 567597C (resistant accessions from Mensah et al., 2005), Dowling, Jackson, PI 71506 (resistant cultivars from Hill et al., 2004), and two susceptible checks, Titan and Williams 82.

In the second study using aphids which had been in colony since 2002 the following soybean genotypes were used: PI 567541B, Dowling, 030108-515 (Resistant), Titan, Roundup-Ready (RR) Titan, E00075, Williams 82 (susceptible).

Location of Experiment and Screening Procedure

The first study was set up as a no-choice (Davis, 1985) test in a factorial experiment arranged in a randomized complete block design with three replications. The two factors in the experiment were the nine soybean genotypes and two aphid colonies (A and B). A total of eight seeds per genotype were planted. In the no-choice test, each

genotype was isolated for the next by the use of a no-see-um mesh cage (Venture Textiles, Inc., Braintree, MA) to prevent the two different sources of aphids from mixing. The second was set up as a Choice test (Davis, 1985) in a randomized complete block design with three replicates. Five plants per genotype were planted. In the choice test the aphids were free to move from plant to plant among genotypes.

Data Collection

Plants were rated visually using a modified version of the method of rating as described by Mensah et al., 2008 (Table 5.1, Appendix) which ranges from 0 for no aphid present to 4 for totally covered with aphids. Data was collected starting from the third week through the fifth. When the susceptible parents first rated a score of 4.0, the data from that sample date were used to classify soybean genotypes as resistant or susceptible. A damage index (DI) for each accession was calculated by the following formula (Zhuang, 1999): DI = Σ(Scale value x No. of plants in the category)/ (4 x Total no. of plants evaluated) x 100. The DI ranges between 0% for no infestation and 100% for the most severe damage. A DI of 38% or less was classified as resistant, whereas a DI of 38% or more was classified as susceptible. The 38% break point was chosen on the basis of the observation that a soybean genotype with a DI value less than 38% never showed symptoms of damage under high aphid pressure until the end of the season.

Statistical Analysis:

Analysis of variance (ANOVA) for choice and no-choice tests was conducted using the PROC GLM procedure in the SAS V9.1 (SAS Institute, 2000). Means were

separated by least significant difference (LSD) at the 5% probability level if their effects were found to be significant in the ANOVA.

RESULTS

Study1

The effects of soybean genotype and the interaction between soybean genotype and aphid colony was found to be significant at P <0.0001 and P=0.0362, respectively. The effect of aphid colony was not significant (P=0.306). All the PIs found to be resistant by Mensah et al., 2005, Jackson and PI 71506, showed antibiosis to both aphid colonies (Table 3.1). However, PI 567543C, and PI 567597C which were formerly reported as having antixenosis resistance was found to exhibit antibiosis resistance in the no choice test. Dowling was found to be susceptible to both aphids from colony A and B. Interestingly, Williams 82 the susceptible check was less susceptible to colony A aphids than Dowling. Titan, the other susceptible cultivar was not significantly different from Dowling (Table 3.1). These results indicate that all the aphids collected randomly in the field in 2006 can overcome the resistant gene *Rag1* in Dowling but not the *Rag* gene in Jackson.

Study 2

In this study the damage index of each entry was calculated at three and then four weeks after inoculation with aphids from the 2002 colony. The results showed that, Dowling was not significantly different from PI 567541B in both rating and both genotypes were resistant. In week three, there was no significant difference between the

four susceptible genotypes Titan, RR Titan, E00075, and Williams 82. However in week four, E00075, the advanced breeding line was significantly less susceptible than Titan, RR Titan, and Williams 82. Over-all the plant damage in week four was not typically different from that in week three (Table 3.2).

DISCUSSION

This research confirms the suspected variation that exists between the soybean aphid populations in Michigan in 2002 and 2006 based on their virulence reaction to Dowling. The results obtained here and that from similar studies carried out in other states show that new soybean aphid biotypes are emerging. This supports the presence of a new soybean aphid biotype in Michigan.

In their recent paper, Kim et al. (2008), report that there are soybean aphid biotypes that can overcome the aphid resistance in both Dowling and Jackson. Another study in Ohio confirms Dowling and Jackson are susceptible to the Ohio isolate of aphids under greenhouse conditions (Mian et al., 2008). In our study Jackson is still resistant to the soybean aphid variant found in Michigan in 2006 (Table 3.1). The breakdown of the resistance in Dowling may have occurred prior to 2006 as a similar trend was observed when some material from Illinois was tested in Michigan in 2005 (personal observation). The report of biotypes occurring in soybean aphids is not unique to this aphid alone. Multiple biotypes have been seen to occur in other aphid species such as Russian wheat aphid and green bug (Burd and Porter, 2006). In the Russian wheat aphid biotype variation was known to exist worldwide, but it was not observed in the U. S. until 2003,

when a biotype was identified that could overcome *Dn4*, the major resistance gene used to protect wheat (*Triticum aestivum* L.) from this aphid (Haley et al., 2004).

There is a need to systematically collect and test soybean aphid isolates in North America and other parts of the world to track potential changes in biotype variation in soybean aphid. This year a project funded and coordinated by Monsanto Company, St. Louis, Mo. will help answer the extent of biotypic variation in soybean aphids in the U.S. In this project all aphid resistant sources would be tested with aphids in states where aphid resistance research is being carried out no-choice tests (Dechun Wang, pers. comm.). DNA-based techniques are increasingly being applied to explore the genetic differences between insect biotypes. These techniques are proving particularly valuable for the study of aphids which are characterized by low levels of intraspecific genetic variation as revealed by allozymes (Hales et al., 1997). For example, restriction analyses of mitochondrial DNA have revealed consistent differences between green bug (*Schizaphis graminum*) biotypes found on different sorghum cultivars (Powers et al., 1989) and between alfalfa aphid (*Therioaphis trifolii*) biotypes using different legume crops (Sunnucks et al., 1997). Additionally, differences in microsatellite profiles have been identified in the English grain aphid (*Sitobion avenae*) collected from wheat (De Barro et al., 1995). Similar molecular work needs to be conducted to determine if indeed soybean biotype diversity exists in North America Based on results of current field studies to detect biotype variation.

In 2005, we reported PI 567543C and PI 567597C as having antixenosis resistance, but our current results (Table 3.1) shows that they possess antibiosis resistance. For a genotype exhibiting antixenosis type resistance the expectation is that

their DI values would be significantly higher than rating obtained for PI567598B and PI 567541B. This difference in resistance mechanism was also observed by Kim et al., (2008) where they classified all the resistance sources from Michigan (Mensah et al., 2005) as showing strong antibiosis. This change in resistance mechanism in PI 567543C and PI 567597C, from antixenosis to antibiosis can be attributed the fact that these aphid biotypes have a different reaction to these two genotypes. It is also possible that the plant defenses have developed antibiotic cues which can now adversely affect the aphid's ability to develop on them.

The identification of new biotypes is of critical importance in accurately identifying genetic sources of resistance crop plants (Smith, 1994). With these reports of soybean aphid biotype diversity emerging, the identification of more effective sources of resistance to aphids is encouraging for resistance breeding efforts. New sources with multiple genetic resistance such as that present in PI 567598B and PI 567541B controlled by two recessive genes (Mensah et al., 2008) are needed to help maintain durability of resistance to soybean aphids.

Table 3.1: The average Damage Index (DI) at 4 weeks after inoculation based on three replications in a no-choice test carried out in Study 1, Fall 2006 the greenhouse.

Entry	Damage Index (%)	
	Colony A	Colony B
PI 567543C	2 a †	0 a
PI 567597C	1 a	2 a
PI 567541B	6 a	0 a
PI 567598B	0 a	0 a
PI 71506	4 a	0 a
Titan	76 bcd	77 bcd
Jackson	0 a	7 a
Dowling	65 bc	67 bc
'Williams 82'	44 b	74 bcd
Mean	22.0	25.2

† Means followed by the same letters in the DI columns are not significantly different by the least significant difference test (P=0.05); Colony A: Dowling Aphids; Colony B: Random 2006 Aphids

Table 3.2: Damage Index (DI) based on results obtained in Study 2-resistant sources tested in the greenhouse, winter 2006 at 3 and 4 weeks after inoculation using aphids originally collected in 2002.

Entry	Damage Index (%)	
	Week 3	Week 4
E00075	75 b†	80 b
030108-515	20 a	20 a
PI 567541B	13 a	13 a
Titan	81 b	88 c
RR Titan	81 b	88 c
Dowling	13 a	13 a
Williams 82	75 b	88 c
Mean	55	51

† Means followed by the same letters in a column are not significantly different by the least significant difference test (P=0.05)

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CHAPTER 4

IDENTIFICATION OF QTLS ASSOCIATED WITH SOYBEAN APHID RESISTANCE IN PI 567598B

ABSTRACT

The soybean aphid (*Aphis glycines* Matsumura) has become a very important pest of soybeans in the U.S. since it was first reported in 2000. Soybean accession PI 567598B, is a source of aphid resistance identified in 2005. The aphid resistance in PI 567598B is controlled by two recessive genes. The objectives of this study were to identify and map quantitative trait loci (QTL) associated with aphid resistance in PI 567598B. One hundred and eighty-eight F_2 individuals randomly selected from a cross between Titan and PI 567598B were genotyped with 109 polymorphic simple sequence repeats (SSR) markers. The F_2 mapping population was screened for aphid resistance in the field in 2005. In 2006 and 2007, the $F_{2:3}$ and $F_{2:4}$ lines were evaluated for aphid resistance in the field. Single marker analysis (SMA) revealed 24 markers on linkage groups (LGs) A2, B2, D1a, D1b, D2, E, G, J, K, M, and O that were significantly ($P \leq 0.05$) associated with aphid resistance. QTL mapping by composite interval mapping (CIM) identified a QTL on LG J that explained from 22 to 32.5 % of the phenotypic variation in the field. The SSR markers flanking these resistance genes can be used in marker-assisted selection for aphid resistance in soybean breeding programs.

INTRODUCTION

Soybean aphids were first reported in the USA in July 2000. Since that time the insect has rapidly spread to the major soybean production areas in the USA and Canada (Plant Health, 2004). Plant damage occurs when large numbers of aphids remove significant amounts of water and nutrients as they feed on leaves and stems, causing leaves to wilt, curl, yellow, and even leaf drop (DiFonzo and Hines, 2002). Yield losses caused by soybean aphid were over 50% in Minnesota in severely infested fields in 2001 (Ostlie, 2002) and up to 52% in China (Wang et al., 1994).

Many soybean breeding programs in the U.S. are currently conducting research on aphid resistance. To date only three programs have successfully found resistance to the soybean aphid, determined the inheritance of resistance and mapped the location of the resistance gene(s). Single dominant genes were found to control resistance to the soybean aphid in the cultivars 'Dowling' and 'Jackson' (Hill et al., 2004, 2006a, 2006b). The gene in Dowling was named as *Rag1* (Hill et al., 2006a). *Rag1* and the resistance gene (*Rag*) in Jackson were mapped to a similar genomic region of linkage group (LG) M using SSR markers (Li et al. 2007). Mensah et al. (2005) identified four soybean accessions among 2,147 with aphid resistance. Accessions PI 567541B and PI 567598B have been shown to be controlled by two recessive genes. Two QTLs controlling the aphid resistance in PI 567541B have been mapped to LGs F and M, respectively (Zhang et al., 2008). Three new soybean aphid resistant accessions have been published recently, PI 243540, PI 567301B and PI 567324 (Mian et al., 2008). In a report to the USDA, Mian and Redinbaugh (2007) reported using SSR markers to map a gene for aphid resistance

that is different from that in the cultivars Dowling and Jackson. With the current reports of soybean aphid biotype diversity in North America (Kim et al., 2008, Mensah et al., 2007, Main et al., 2008), there is the need to map the location of more soybean aphid resistant genes so that markers flanking them can be used in marker assisted selection and gene pyramiding. The use of conventional breeding alone would delay the release of aphid resistant soybeans. During the last few years, molecular marker aided-selection has been used successfully for the breeding of crops with improved quantitative traits (Dubcovsky, 2004). Identification of molecular markers that are closely linked to the aphid resistance genes in PI 567598B would enable the use of marker-assisted selection for aphid resistance in segregating populations and would facilitate the incorporation of the resistance genes into adapted northern soybean breeding lines. Over 1,000 SSR markers have been mapped to an integrated genetic linkage map of the soybean (Song et al., 2004) and these markers have been successfully employed in marker-assisted selection by many breeding programs. The objective of this study is to map the aphid resistance genes in PI 567598B and to identify flanking markers that could be used in marker-assisted selection.

MATERIALS AND METHODS

Plant Materials

A population of 388 F_2 individuals developed from a single F_1 plant from a cross between Titan, an aphid susceptible cultivar, and PI 567598B were used for QTL detection. The initial cross was made in the summer of 2003. A total of one hundred and

eighty-eight resistant and susceptible F_2 individuals were randomly selected for use as the mapping population. The 188 individuals were advanced to $F_{2:3}$ and $F_{2:4}$ in 2006 and 2007 respectively.

Field Planting, Inoculation and Data collection

Three hundred and eighty-eight F_2 seeds and the two parents of the cross between Titan and PI 567598B were planted in a 12.2- x 18.3-m aphid-proof polypropylene cage with the 0.49-mm size mesh (Redwood Empire Awning Co., Santa Rosa, CA), in the field at the Agronomy Farm, MSU, East Lansing, MI in June 2005. Two weeks after planting, when the plants were at the V2 stage (Fehr and Caviness, 1977), each plant was inoculated by gently placing two wingless aphids with a camel hair brush on the newly emerged trifoliate (Mensah et al., 2005). Aphids used in this study were collected from natural colonies in nearby naturally infested soybean fields. The F_2 plants were planted approximately 2 cm apart and the parents were planted 5.1 cm apart with two replications. The F_2 and parental plants were rated for aphid damage two, three, and four weeks after inoculation using a modified version of the rating scale of 0 to 4 described by Mensah et al., (2005) (Table 5.1, appendix). Data collected at weeks 3 and 4 were used to identify DNA markers associated with aphid resistance.

In 2006 and 2007, depending on seed availability, up to twelve $F_{2:3}$, $F_{2:4}$ seeds per family and parents were planted in the summer of each year. The aphids used for inoculation were collected from naturally occurring colonies in the field in the respective years. Inoculation and data collection were conducted as described above for the F_2 population. The aphid resistance score was determined as the mean of the scored plants in each line.

DNA Extraction and Marker Analysis

The unopened trifoliate from each individual was harvested from each F_2 plant in the field and kept on ice. The leaf samples were kept at -80°C for 24 hours and then lyophilized for approximately 72 hours. The DNA was extracted with the CTAB (hexadecyltrimethyl ammonium bromide) method described by Kisha et al. (1997). The population and parents were genotyped using SSR marker pairs. The SSR primer sequences were obtained according to Song et al. (2004). A total of 1059 SSR markers were screened for polymorphism between the two parents. The DNA amplification of SSR markers was performed using 15µl polymerase chain reaction (PCR) consisting of 1.0 x PCR buffer (10 mM Tris-HCl, 50 mM KCl, 0.01% Gelatin, pH=8.3), 3.0 mm $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP, and dTTP (Sigma-Aldrich, St. Louis, MO), 0.3 µM each of forward and reverse primers, 100 ng of genomic DNA and 1 unit of *Thermus acquaticus (Taq)* DNA polymerase. The PCR amplification conditions consisted of an initial denaturing step of 94°C for 4 min, followed by 43 cycles of 25 sec. of denaturing at 94°C, 25 sec. of annealing at 47°C, and 25 sec. of extension at 68°C, with a final seven minute extension at 72°C before cooling down to 4°C in a MJ Tetrad™ thermal cycler (MJ Research, Waltham, MA). Gel electrophoresis was performed using non-denaturing polyacrylamide gels as described by Wang et al. (2003). After electrophoresis, gels were photographed under UV light and scored. The SSR markers were scored co-dominantly as 'a' = homozygous for the marker allele from the resistant parent, 'h' = heterozygous for the marker, or 'b' = homozygous for the marker allele from the susceptible parent. Situations where it was difficult to distinguish 'a' and 'h'

were scored as'd' and those where 'b' and 'h' could not be distinguished were scored as 'c'.

Linkage Map Construction

Analysis of linkage between the aphid resistance genes and associated SSR marker loci, and calculation of their relative map positions, was performed with JoinMap 3.0 (Van Ooijen and Voorrips 2001) using the Kosambi mapping function. A logarithm (base 10) of the odds (LOD) score of 3 or higher was used to identify those loci linked to aphid resistance. The best position of each marker was searched by comparing the goodness-of-fit for each tested position to determine the order and distance among markers within each linkage group.

QTL Analysis

Analysis of variance (ANOVA) was performed for phenotypic data from the field data using the GLM procedure of SAS (1999). QTL analysis was performed in WinQTL Cartographer V2.5 (Wang et al., 2005). The trait data used in the analysis was the aphid rating scores at three and four weeks after inoculation. Single marker analysis was performed and markers showing significance ($p \leq 0.05$) were identified. Composite interval mapping (CIM) was performed to detect QTLs for aphid resistance using QTL Cartographer V2.5 with the standard model Zmapqtl 6. The CIM analysis uses markers other than the interval being tested as cofactors to control the genetic background (Zeng, 1994). The forward and backward regression method was used to select markers as

cofactors. The walk speed chosen for CIM was 2 cM and a window size of 10 cM. The empirical LOD threshold at 5% probability level was determined by a 1,000-permutation test (Churchill and Doerge, 1994). QTLs were graphically displayed with line maps using MapChart (Voorrips, 2002).

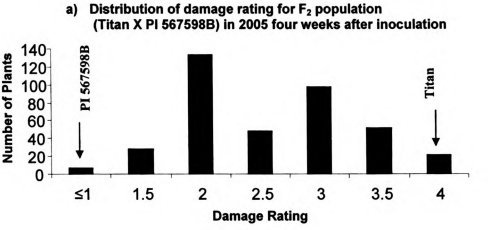
RESULTS

Phenotypic Data

Titan and PI 567598B consistently showed significant difference in aphid resistance in each trial for both three-week and four-week ratings. The resistant parent PI 567598B always had a significantly ($P < 0.05$) lower score than Titan. In general, the four-week rating score was higher than the three-week rating score for the same line in all three years of evaluation (Table 5.2, Appendix). The damage rating for the F_2 population (Titan x PI 567598B) showed continuous variation and approximately normal distribution, suggesting that aphid resistance is a quantitative trait controlled by multiple genes and ranged from 0.5 to 4 (Fig 4.1 a). From within this population all the resistant lines and random selection of susceptible lines were chosen to generate the mapping population of 188 individuals. The phenotypic data for the mapping population was approximately normally and continuously distributed in all three years for data collected four weeks after inoculation (Fig. 4.1 c, e and g). This indicates that more than one recessive gene may control aphid resistance in PI 567598B. The data collected three weeks after inoculation in 2005 and 2006 was skewed towards the resistant parent and ranged from 0 to 3 (Fig.4.1 b and d). In 2007, three weeks post inoculation, the lines

were quite evenly distributed over the lower part of the rating scale and ranged from 0.5 to 3.5 (Fig.4.1e).

Figure 4.1


a) Distribution of damage rating for F2 population
(Titan X PI 567598B) in 2005 four weeks after inoculation
Number of Plants
140
120
100
80
60
40
20
0
PI 567598B
Titan
≤1
1.5
2
2.5
3
3.5
4
Damage Rating

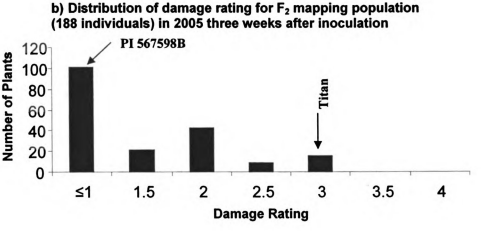
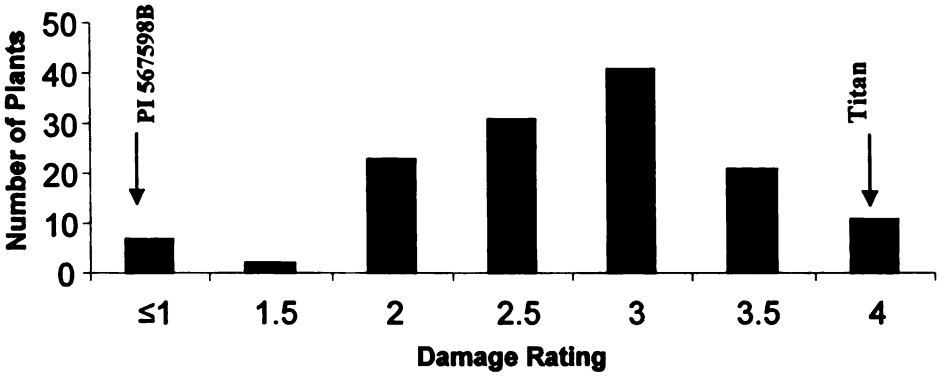

b) Distribution of damage rating for F2 mapping population
(188 individuals) in 2005 three weeks after inoculation
Number of Plants
120
100
80
60
40
20
0
PI 567598B
Titan
≤1
1.5
2
2.5
3
3.5
4
Damage Rating

Fig 4.1 (cont'd)

c) Distribution of damage rating for F_2 mapping population (188 individuals) in 2005 four weeks after inoculation



d) Distribution of damage rating for $F_{2:3}$ mapping population (188 individuals) in 2006, three weeks after inoculation

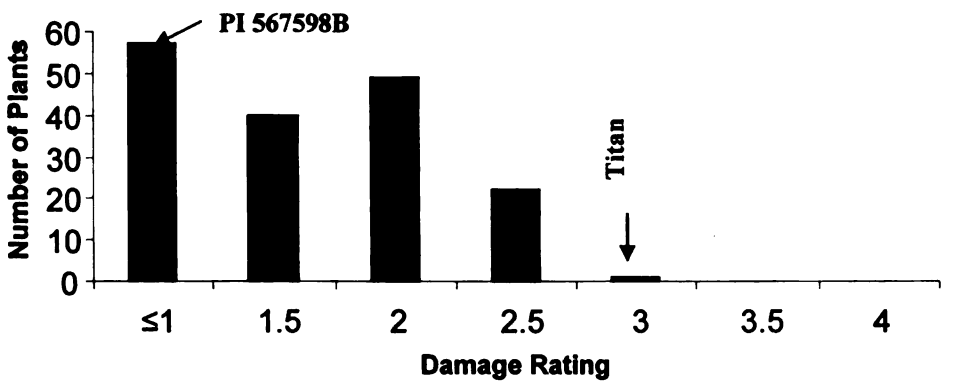
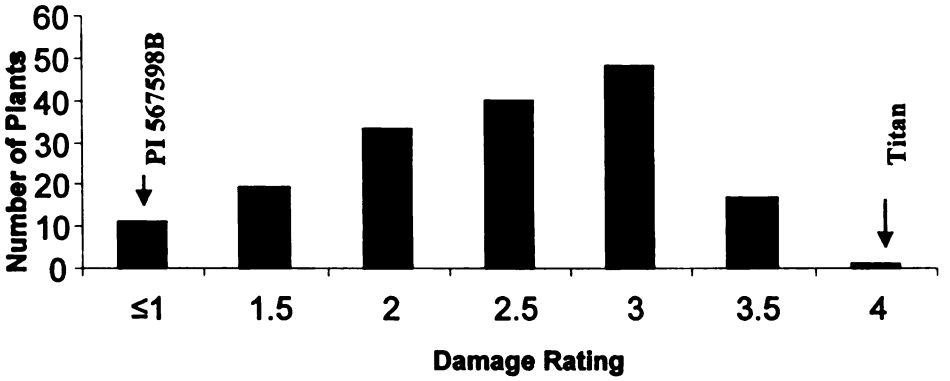


Figure 4.1 (cont'd)

e) Distribution of damage rating for $F_{2:3}$ mapping population (188 individuals) in 2006, four weeks after inoculation



f) Distribution of damage rating for $F_{2:4}$ mapping population (188 individuals) in 2007, three weeks after inoculation

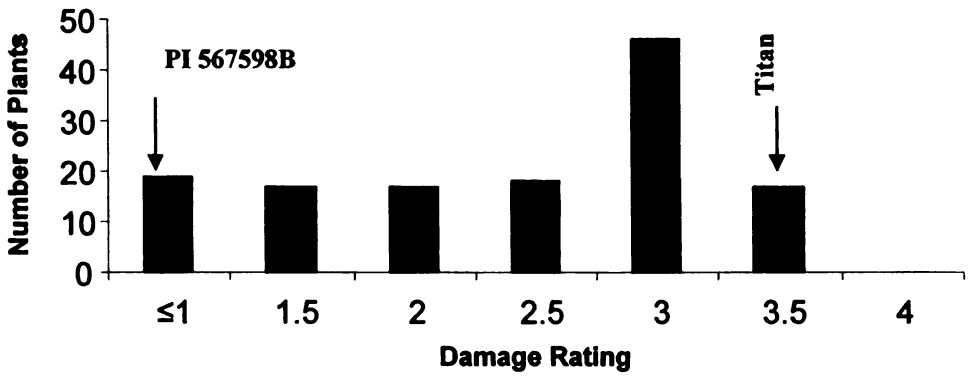


Figure 4.1 (cont'd)

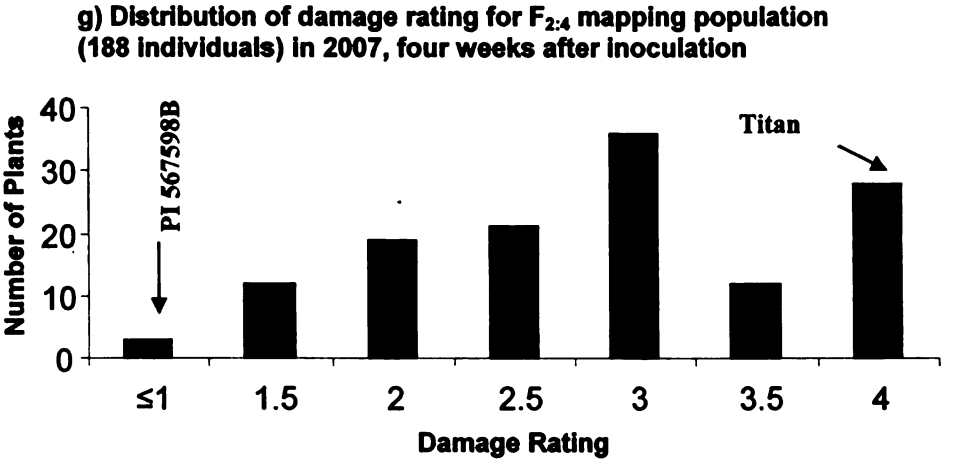


Figure 4.1: The damage rating distributions of: a) the F_2 population of the cross between Titan and PI 567598B; b-g) 188 individuals of mapping population for 2005, 2006 and 2007 three and four weeks after inoculation.

Identification of QTL for Soybean Aphid Resistance

Of the 1050 SSR markers tested for polymorphism between the two parents, only 109 that were polymorphic, easy to score and had good amplification were used to genotype the mapping population. Out of 109 polymorphic markers 58 were placed into 23 linkage groups that were segments of the 20 linkage groups on the consensus map by Song et al. (2004). The remaining markers were unanchored but had some markers significantly associated with aphid resistance from SMA. The total map distance of the 23 linkage groups was 760 cM, with an average interval length of 10.8 cM and covering 38% of the soybean genome.

In the SMA, 24 markers were found to be significantly associated with aphid resistance in both years ($p \leq 0.05$). In 2005, at three and four weeks after inoculation ten and nine markers respectively were significantly associated with aphid resistance with four significant in both weeks. Six markers were significantly associated with resistance in 2006. Satt529 and Satt171 were significant in both weeks three and four weeks after inoculation. In 2007, seven and six markers were associated with aphid resistance three weeks and four weeks after inoculation respectively. Five markers were significantly associated with aphid resistance in both weeks. Only Satt280 and Satt529 on LG J were consistently associated with aphid resistance in 2005, 2006 and 2007 (Table 4.1, 4.2). In the CIM analysis, empirical significance threshold was computed as LOD score of 5.05 using 1000 permutations in the mapping population in 2005. However, none of markers that were significantly associated aphid resistance in 2005 had a LOD score equal or greater than 5.05. The putative QTL on LG J had the highest LOD score of 4.17 (Fig.

4.2A) and accounted for 23.0% of aphid resistance variation three weeks after inoculation in 2006. This putative QTL on LG J was also observed in 2007 week 4 with a LOD of 3.08 and an R^2 of 32.5 % (Fig. 4.2 A). Another putative QTL (LOD = 4.19) and R^2 of 46.6% was detected on LG C1 in 2005, three weeks after inoculation close to the SSR marker Sat_178 (Fig.4.2C). In 2007, CIM analysis the empirical significance threshold computed was 4.65 after 1000 permutations. The only QTL identified in 2007 was in week 4 with a LOD of 3.29 and R^2 of 22.0% (Fig. 4.2B) and significant in SMA at $p<0.0001$ (Table 4.1, 4.2). This putative QTL on LG J was closer to Satt529.

Table 4.1: Markers significantly associated with soybean aphid resistance in PI 567598B in single marker analysis in 2005, 2006 and 2007 at three weeks after inoculation.

Marker	Linkage Group	Position (cM)	2005 week3	2006 week3	2007 week3
Satt341	A2	77.69	0.007**	NS	NS
Satt304	B2	65.55	0.024*	NS	NS
Sat_355	B2	66.23	0.036*	NS	NS
Satt070	B2	72.81	NS	NS	0.011*
Satt321	D1a	50.16	NS	NS	NS
Satt095	D1b	25.60	0.015*	NS	NS
Satt005	D1b	75.29	NS	NS	NS
Satt271	D1b	137.05	NS	NS	NS
Satt208	D2	67.91	NS	NS	NS
Satt699	E	41.24	NS	NS	NS
Satt685	E	56.69	NS	NS	0.010*
Satt163	G	0.00	NS	NS	0.025*
Satt280	J	38.70	0.011*	0.286	0.032*
Satt686	J	40.50	0.019*	NS	NS
Satt529	J	41.29	0.004**	0.011*	0.014*
Satt285	J	25.51	NS	NS	NS
Satt380	J	43.11	NS	NS	0.016*
Satt215	J	44.81	NS	NS	NS
Satt628	K	49.59	NS	NS	NS
Satt273	K	56.62	NS	NS	0.039*
Satt435	M	38.94	NS	0.044*	NS
Sat_038	O	112.17	0.05*	NS	NS
Satt216	-		NS	NS	0.352
Satt171	-		NS	0.009**	NS

NS= not significant 0.05 probability level. Markers significant at 5%, 1%, 0.1% and 0.01% levels are indicated by *, **, ***, and **** respectively. Linkage group names and relative position for the markers were assigned according to the Soybean Composite Map (Song et al., 2004).

Table 4.2: Markers significantly associated with soybean aphid resistance in PI 567598B in single marker analysis in 2005, 2006 and 2007 at four weeks after inoculation.

Marker	Linkage Group	Position (cM)	2005 week4	2006 week4	2007 week4
Satt341	A2	77.69	NS	NS	NS
Satt304	B2	65.55	0.012*	NS	NS
Sat_355	B2	66.23	NS	NS	0.684
Satt070	B2	72.81	NS	NS	0.112
Satt321	D1a	50.16	NS	0.027*	NS
Satt095	D1b	25.60	0.005**	NS	NS
Satt005	D1b	75.29	NS	NS	NS
Satt271	D1b	137.05	0.024*	NS	NS
Satt208	D2	67.91	0.008**	NS	NS
Satt699	E	41.24	NS	0.012*	NS
Satt685	E	56.69	0.053	0.024*	0.034*
Satt163	G	0.00	0.024*	NS	0.026*
Satt280	J	38.70	0.008**	NS	0.006**
Satt686	J	40.50	NS	NS	NS
Satt529	J	41.29	0.001**	0.004**	0.000****
Satt285	J	25.51	0.001***	NS	0.114
Satt380	J	43.11	NS	NS	0.020*
Satt215	J	44.81	0.030*	NS	NS
Satt628	K	49.59	0.012*	NS	NS
Satt273	K	56.62	NS	NS	0.492
Satt435	M	38.94	NS	NS	NS
Sat_038	O	112.17	NS	NS	0.083
Satt216	-	-	0.021*	NS	0.011*
Satt171	-	-	NS	0.003**	NS

NS= not significant 0.05 probability level. Markers significant at 5%, 1%, 0.1% and 0.01% levels are indicated by *, **, ***, and **** respectively. Linkage group names and relative position for the markers were assigned according to the Soybean Composite Map (Song et al., 2004).

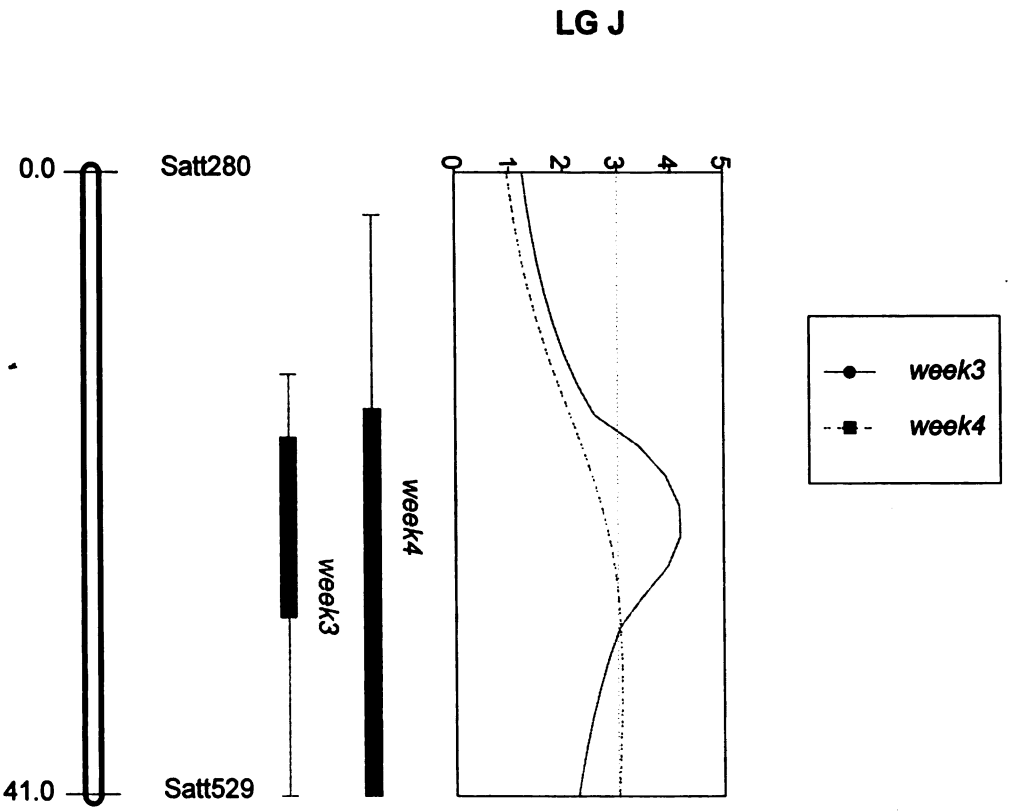


Figure 4.2A: Putative QTLs associated with Aphid resistance on linkage group J based on phenotypic data from 2005 week3 and week4 data. The map distances between the markers are given in cM (centimorgans). The linkage groups are named according to Song et al. (2004). The LOD threshold was set at 3.0.

LGJ

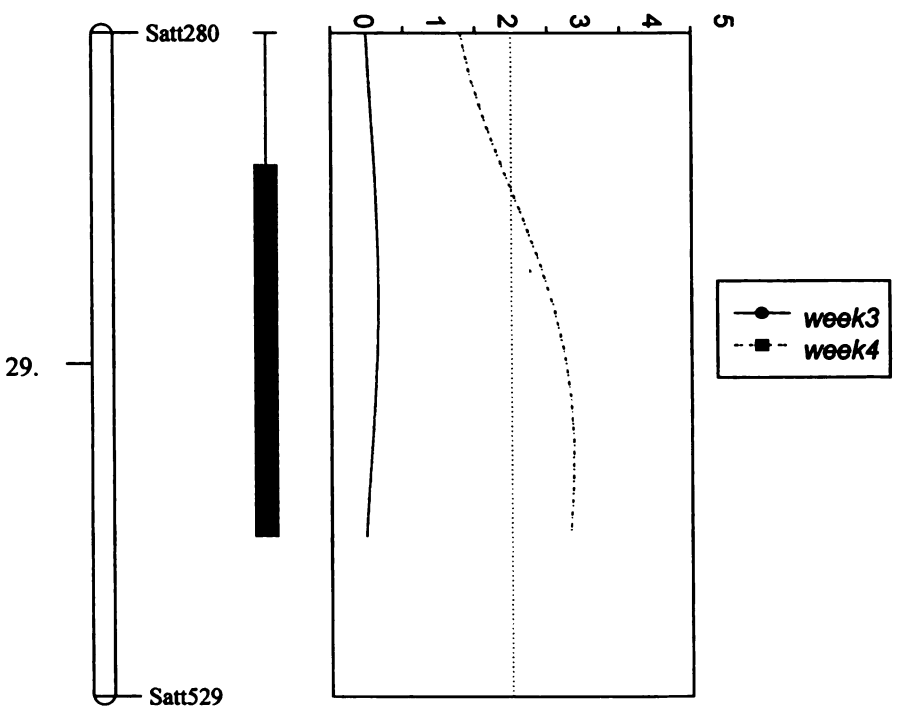


Figure 4.2 B: Putative QTL associated with Aphid resistance on linkage group J based on phenotypic data from 2007 week 3 and week4 data. The map distances between the markers are given in cM (centimorgans). The linkage groups are named according to Song et al. (2004). The LOD threshold was set at 3.0.

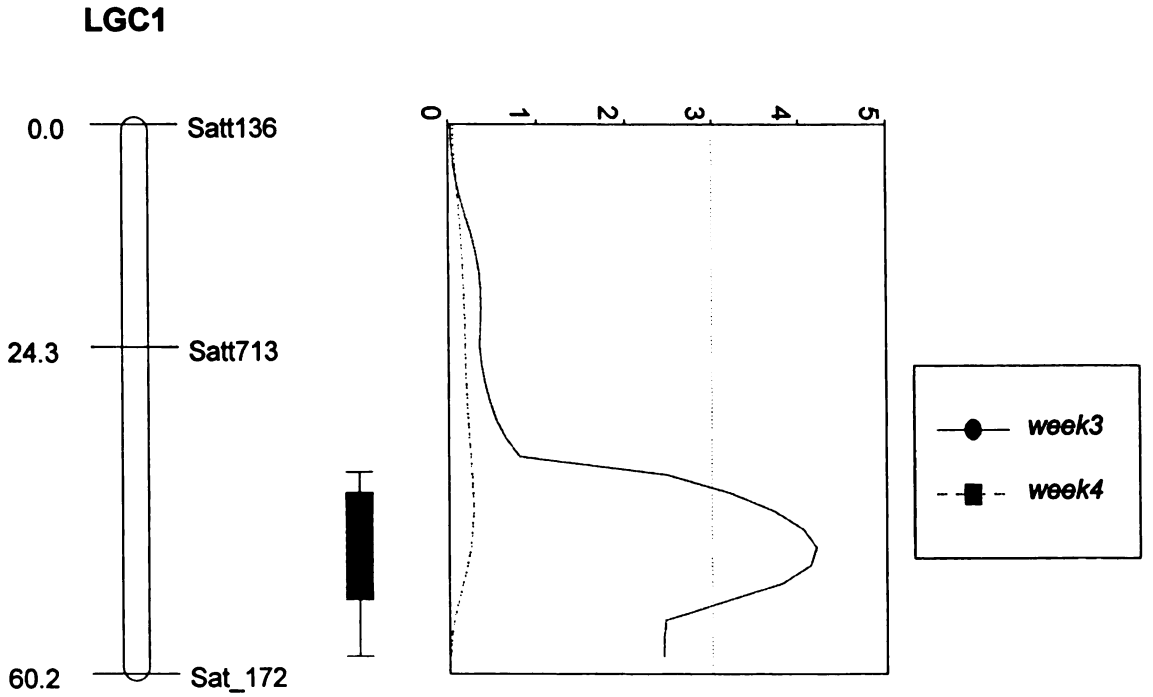


Figure 4.2 C: Putative QTL associated with Aphid resistance on linkage group C1 based on phenotypic data from 2005 week3 data. The map distances between the markers are given in cM (centimorgans). The linkage groups are named according to Song et al. (2004). The LOD threshold was set at 3.0.

DISCUSSION

Markers associated with soybean aphid resistance were detected in all three years of this study. The markers found to be significantly associated with aphid resistance in PI 567598B have not been previously reported. The markers Satt280 and Satt529 on LG J were consistently associated with aphid resistance in SMA in all three years and at two screening dates. In all but one year (2006) and one data collection time (2005 week3), a putative QTL was identified on LG J between Satt280 and Satt529. The putative QTL on LG J was also tested in 44 resistant lines derived from a cross with PI 567598B as the resistant parent but in different genetic backgrounds. Both markers flanking the QTL were found to be associated with resistance in all 44 lines (Menghan Liu, pers. Comm.).

The first report of markers associated with aphid resistance (Li et al., 2007), using SSR markers and data for aphid resistance from $F_{2:3}$ populations developed from crosses between Dowling and the two susceptible soybean cultivars 'Loda' and 'Williams 82', and between Jackson and Loda. The resistance genes *Rag1* (Hill et al., 2006a) and *Rag* from cultivar Jackson segregated 1:2:1 in the $F_{2:3}$ populations and mapped to the same location on LG M between the markers Satt435 and Satt463. This suggests that the two resistance genes maybe allelic or tightly linked. The markers associated with aphid resistance in PI 567541B (Mensah et al., 2005), have been recently mapped using a population of 228 recombinant inbred lines (RILs). Using CIM two, QTLs controlling the aphid resistance in PI 567541B were found on LGs F and M, respectively (Zhang et al., 2008). Mian and Redinbaugh, (2007) reported using SSR markers to map a gene for aphid resistance that is different from the aphid resistance genes from cultivars Dowling

and Jackson (Hill et al, 2004). This resistance gene is from a new aphid resistance source described in Mian et al., (2008).

The QTLs found in this study will assist breeders in marker-assisted selection (MAS) when breeding for aphid resistance using PI567598B. Currently, MAS is being used in breeding for soybean aphid resistance, using the markers flanking *Rag1* and *Rag* genes to accelerate the breeding process and reduce the cost associated with aphid resistance bioassays. After a year and a half of MAS, aphid resistant $BC3F_2$ lines containing *Rag1* have been released to public and private soybean breeders (Li et al., 2007).

The initial expectation was to identify two QTLs associated with aphid resistance in PI567598B corresponding to the two recessive genes controlling aphid resistance in the germplasm accession. During the QTL mapping of aphid resistance in this population one big challenge encountered was the surprisingly low number of polymorphic markers between Titan and PI 567548B. Being a wide cross the expectation was to have more polymorphic markers but this was not observed due to technical difficulties encountered while screening for polymorphism. Only one locus was mapped and may be attributed to the mapping population being used was on an individual plant basis. To correct this situation an $F_{4:5}$ populations of the same cross (Titan x PI567598B) was phenotyped in the greenhouse and genotyped with markers found in this study to be associated with aphid resistance. Only the QTL on LG J was detected. In the study by Zhang et al., 2008, they found that the two resistant genes in PI 567541B were expressed differently between field and greenhouse trials. Only one gene was expressed in the greenhouse while both genes are expressed in the field.

The results from this study will guide our future investigation of QTLs underlying aphid resistance in PI 567598B. Less than 10% of the available SSR markers were mapped and many of them were unlinked, therefore more marker data needs to be obtained. In the future careful consideration must be given to the type of mapping population chosen for different patterns of inheritance. The knowledge of markers associated with aphid resistance in different sources is very essential as it will be useful in gene pyramiding (Mittal et al., 2008). Combining different sources of aphid resistance is important to develop durable management programs, especially with the rapid development of new aphid biotypes in response to resistance gene deployment (Kim et al., 2008, Mensah et al. 2007). Although these reports are the first few evidence of biotypes of soybean aphids in North America, in the Russian wheat aphid many biotypes have arisen in response to the deployment of aphid resistance genes (Harvey et al., 1997, Haley et al., 2004). Gene pyramiding would be useful to introduce genes for resistance to multiple biotypes of the soybean aphid, as and when new sources of resistance are identified in different environments. Combining aphid resistance genes may decrease the problem of aphids overcoming resistance since the pest would need to deal with each resistance gene. With the resistance genes in PI 567541B, PI 567548B, Dowling and Jackson being different this is a very good opportunity to stack these genes as new biotypes evolve.

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APPENDIX

Table 5.1: Visual rating scale used to establish the Damage Index (DI) of a plant.

Damage Rating	Number of Aphids	Aphid Colony	Plant Characteristics
0.0	0	-	Normal and healthy
0.5	≤ 10	No colony	Normal and healthy
1.0	≤ 100	Young leaves	Normal and healthy
1.5	101-150	Young leaves	Normal and healthy
2.0	151-300	Young leaves and tender stem	Normal and healthy
2.5	301-500	Both young and old leaves, undersides and tender stem	Normal and healthy
3.0	501-800	Both young and old leaves, undersides of leaves and hard stems	Leaves shiny, slightly curled,
3.5	≥800	On all leaves and stems, few cast skins	Leaves curled and slightly yellow, plants stunted, no sooty mold,
4.0	≥800	On all leaves and stems many cast skins	plants stunted, leaves severely curled, yellow, covered with sooty mold

Table 5.2: Phenotypic data for 188 individuals of mapping population [F_2 (2005), $F_{2:3}$(2006) and $F_{2:4}$(2007)] collected three and four weeks after inoculation.

	2005 F_2†		2006 $F_{2:3}$‡		2007 $F_{2:4}$‡	
Individual	Week 3	Week 4	Week 3	Week 4	Week 3	Week 4
1	1.5	2	0.7	1.5	0.7	1.1
2	2	3.5	0.7	1.8	-	-
3	2.5	3.5	1.0	2.1	1.1	1.5
4	3	3	1.0	2.4	-	-
5	2.5	3	0.7	2.7	1.6	1.8
6	2.5	3	-	-	-	-
9	2	3.5	2.5	3.0	2.6	2.0
10	2	4	1.7	2.3	1.1	1.6
11	3	3.5	0.8	2.0	1.6	3.0
12	2.5	3	2.0	3.0	2.7	3.0
13	2.5	3	0.7	1.0	0.5	2.5
14	2	3	1.2	1.5	-	-
17	1	2	0.5	0.7	0.5	1.2
18	3	3.5	1.5	2.0	1.7	2.8
19	2	3	2.0	2.0	2.0	2.0
20	1	2.5	1.5	1.5	1.9	2.8
22	2.5	3.5	0.9	1.2	1.7	2.9
24	2	3	1.0	1.5	0.8	1.1
27	2.5	3.5	1.3	1.8	1.1	3.0
28	1	2	0.9	1.0	1.4	2.5
32	1	2	1.3	1.8	1.5	2.4
33	1.5	2	1.0	2.0	0.8	2.3
34	1	2.5	2.0	3.0	3.0	3.6
37	2	3	-	-	3.0	3.8
38	1	2	2.5	3.0	2.5	2.0
40	1	2	1.0	1.2	0.7	1.9
43	1	2	0.8	1.3	1.2	1.0
44	1	2	0.8	1.0	1.5	2.9
45	1.5	2.5	1.0	1.3	2.7	2.0
46	1	1.5	1.0	0.9	1.8	2.3
47	1	2	1.5	2.3	1.9	2.0
49	2	3.5	2.5	3.5	2.5	4.0
51	1	2	1.0	2.2	-	-
52	1	2.5	2.3	3.0	3.5	3.0
53	1	2.5	2.0	2.5	2.5	2.5
55	1	3	-	-	-	-
56	2	2.5	2.6	3.5	-	-
57	1	1.5	-	-	-	-
58	1	1.5	-	-	-	-
59	2	3.5	2.0	2.8	2.7	3.5
61	1	1.5	2.5	2.8	3.0	3.5
62	1	1	2.0	2.5	-	-
66	0.5	2.5	2.0	2.9	2.7	3.3

†: Damage rating of individual F_2 plant, ‡: Mean Damage rating for up to 15 plants

Table 5.2 cont'd

	2005 F_2†		2006 $F_{2:3}$		2007 $F_{2:4}$	
Individual	**Week 3**	**Week 4**	**Week 3**	**Week 4**	**Week 3**	**Week 4**
67	0	3	-	-	-	-
68	1	2.5	2.5	3.0	3.5	4.0
70	0	1	1.3	1.9	3.0	4.0
73	1	2	2.5	2.9	3.3	2.7
74	2	2.5	2.0	2.5	-	-
75	2	2.5	1.0	1.2	1.4	2.1
77	1	2.5	1.2	2.0	2.0	3.0
80	1.5	2	1.5	2.3	0.5	0.9
81	1.5	2.5	1.7	2.7	1.0	1.2
83	2	3	1.0	2.2	1.4	2.0
84	1	2	1.5	2.9	2.5	3.0
85	1	1.5	2.5	3.2	2.8	4.0
86	1	1.5	2.0	2.4	2.1	3.7
87	3	3.5	2.1	2.6	2.7	4.0
88	3	4	2.5	3.0	3.5	4.0
89	1	1.5	-	-	-	-
92	0.5	1	-	-	-	-
93	1	2.5	2.0	2.6	2.0	2.3
94	0.5	1	2.5	3.3	2.0	2.0
95	0.5	1	0.5	1.0	0.5	1.0
96	1	2	1.0	1.9	1.0	2.5
98	2	2.5	2.0	3.2	2.0	3.4
99	1	1.5	2.0	2.4	1.0	2.0
100	1	2.5	1.5	2.7	2.8	4.0
101	1	3.5	2.0	3.1	-	-
102	1	2	1.5	1.2	0.5	4.0
103	1	2	2.0	2.5	3.0	4.0
105	3	4	2.5	3.2	3.1	4.0
106	1	3.5	1.5	1.9	2.0	3.0
107	1.5	2	1.3	1.3	2.6	2.7
108	1	2	2.0	2.6	2.1	4.0
109	1	2.5	1.0	1.7	2.4	3.0
110	1	2.5	1.0	2.2	1.0	1.8
111	1.5	2	2.0	2.8	2.7	3.3
112	2	3	2.0	2.6	-	-
113	2	3	1.0	1.0	2.2	2.8
114	1.5	2.5	1.0	1.8	1.0	1.4
115	2	2.5	1.5	2.2	-	-
116	1.5	2	1.0	1.8	-	-
117	1	2	1.0	2.5	0.5	3.0
118	1	2.5	1.0	2.2	-	-
119	1	1.5	1.0	1.0	-	-

†: Damage rating of individual F_2 plant, ‡: Mean Damage rating for up to 15 plants

Table 5.2 cont'd

	2005 F_2†		2006 $F_{2:3}$‡		2007 $F_{2:4}$‡	
Individual	**Week 3**	**Week 4**	**Week 3**	**Week 4**	**Week 3**	**Week 4**
121	1.5	2	1.0	2.2	2.1	2.5
122	1	2	0.5	1.3	1.5	2.1
123	1.5	2	1.0	2.5	-	-
124	1	2	1.5	2.5	-	-
126	1	1.5	-	-	-	-
128	3	4	2.5	3.0	3.5	4.0
129	3	4	2.5	3.0	3.0	4.0
131	1	2	0.5	1.5	-	-
132	3	3.5	2.3	3.1	-	-
133	3	3.5	1.3	1.5	2.2	2.1
135	1	1.5	2.0	3.0	2.4	2.8
137	1	2.5	1.0	2.0	2.7	2.5
138	1	2.5	1.0	2.2	2.5	4.0
139	2	3	1.0	2.3	-	-
140	3	4	2.0	3.0	-	-
142	2	2.5	2.0	3.3	3.5	4.0
144	2	3	2.0	2.9	3.5	3.8
145	2	3.5	2.0	2.6	2.4	3.0
147	1	2	2.5	3.5	3.3	2.3
149	1	2	2.0	2.8	3.4	4.0
150	0.5	1.5	1.5	1.5	2.7	3.3
151	2	3	1.0	2.4	3.4	1.8
154	1	2	-	-	-	-
157	1	1.5	1.2	1.3	1.0	1.5
158	1	1.5	1.0	1.0	1.5	1.2
159	0.5	1.5	-	-	-	-
160	3	4	2.5	3.5	3.5	4.0
163	2	3	1.5	2.2	2.7	3.0
164	1	1.5	1.0	1.3	2.2	2.9
165	1	2.5	2.0	3.2	3.0	3.0
169	1	3	1.0	1.0	-	-
170	0.5	2.5	-	-	-	-
175	1	2	2.0	3.0	-	-
177	0.5	1.5	2.0	2.7	3.0	3.5
181	0.5	3	1.9	2.4	2.8	3.0
182	1	3	1.5	2.1	3.0	3.0
183	2	3	1.5	2.5	-	-
184	2	3	1.0	2.7	-	-
187	1	2	2.0	2.8	3.5	3.0
188	1	3	1.5	2.1	2.9	4.0
189	2.5	4	2.0	3.2	3.0	3.0
190	3	4	-	-	-	-
191	2	3	2.0	2.5	-	-

†: Damage rating of individual F_2 plant, ‡: Mean Damage rating for up to 15 plants

Table 5.2 cont'd

	2005 F_2†		2006 $F_{2:3}$‡		2007 $F_{2:4}$‡	
Individual	**Week 3**	**Week 4**	**Week 3**	**Week 4**	**Week 3**	**Week 4**
192	2	3	1.5	2.0	2.5	3.5
193	2	3	1.2	2.3	1.5	3.0
195	1.5	2.5	1.0	2.1	2.4	2.5
198	1	2	1.0	1.9	1.0	2.0
199	1	2	2.2	2.7	3.0	1.6
200	1	1.5	-	-	-	-
201	2	3.5	1.8	2.7	2.7	4.0
203	1	2	2.0	2.6	1.7	2.5
204	1	2	-	-	-	-
207	1	1.5	1.5	1.3	3.0	1.5
213	1	1	-	-	-	-
214	1	1	1.5	2.3	2.0	1.5
216	1	1.5	-	-	-	-
217	1	2	1.8	2.7	1.5	2.0
218	1	2	2.0	3.0	2.8	2.0
231	1	2	1.3	1.8	2.9	2.5
235	1	3	2.0	2.7	2.5	3.5
236	1.5	3	2.0	2.7	2.7	3.0
237	1.5	3	1.2	2.2	3.0	4.0
239	1	2	2.0	2.7	3.2	3.5
240	1	2	1.0	2.0	1.0	2.0
242	1	2	0.8	1.9	3.0	3.0
247	1	2	1.3	1.9	2.7	3.0
249	1	2.5	1.0	2.5	2.0	3.0
250	1	2	2.0	2.8	3.0	2.5
251	1	1.5	2.0	3.0	3.5	3.5
253	1	2	2.0	3.1	-	-
255	2	3.5	2.0	3.0	3.0	3.0
258	2	3	1.5	1.9	2.6	3.0
265	2.5	3.5	1.5	2.0	3.0	3.0
266	2	3.5	2.0	3.0	-	-
267	2	3	1.0	2.5	2.8	2.5
268	2	3	1.0	1.9	3.0	4.0
270	1.5	3	1.0	2.2	-	-
274	3	4	1.0	1.9	-	-
293	2	3	2.0	3.0	1.5	1.5
302	2	3	2.0	2.8	3.0	3.0
306	1.5	3	2.5	3.2	3.5	4.0
327	1	1.5	1.0	1.8	1.2	2.4
329	1	2	2.3	2.8	-	-
349	1	3	1.0	2.5	1.5	2.0
355	1.5	3	1.5	1.9	-	-
386	1.5	2.5	1.0	2.4	-	-

†: Damage rating of individual F_2 plant, ‡: Mean Damage rating for up to 15 plants

Table 5.2 cont'd

	2005 F_2†		2006 $F_{2:3}$‡		2007 $F_{2:4}$‡	
Individual	**Week 3**	**Week 4**	**Week 3**	**Week 4**	**Week 3**	**Week 4**
387	1	2	1.5	1.8	2.1	1.5
402	2	3.5	1.0	1.8	-	-
403	1.5	2	1.0	1.8	3.5	4.0
404	2	2.5	1.5	2.3	1.0	2.5
407	1.5	2	2.0	2.5	1.5	4.0
503	1	2	1.5	2.6	-	-
505	1	2	1.5	2.0	-	-
508	1	2.5	1.5	2.0	2.9	-
512	1	2	1.5	2.0	2.8	-
519	1.5	2	2.5	4.0	3.0	-
280	2	2.5	2.0	2.8	3	3.0
340	3	4	-	-	-	-
400	2	3.5	1.5	1.5	2	3.0
487	2	3	2.5	3.3	3	3.5
492	2	3	-	-	-	-
521	1	1.5	-	-	-	-
T			2.5	3.0		4.0
N			0.7	1.0		1.5

†: Damage rating of individual F_2 plant, ‡: Mean Damage rating for up to 15 plants

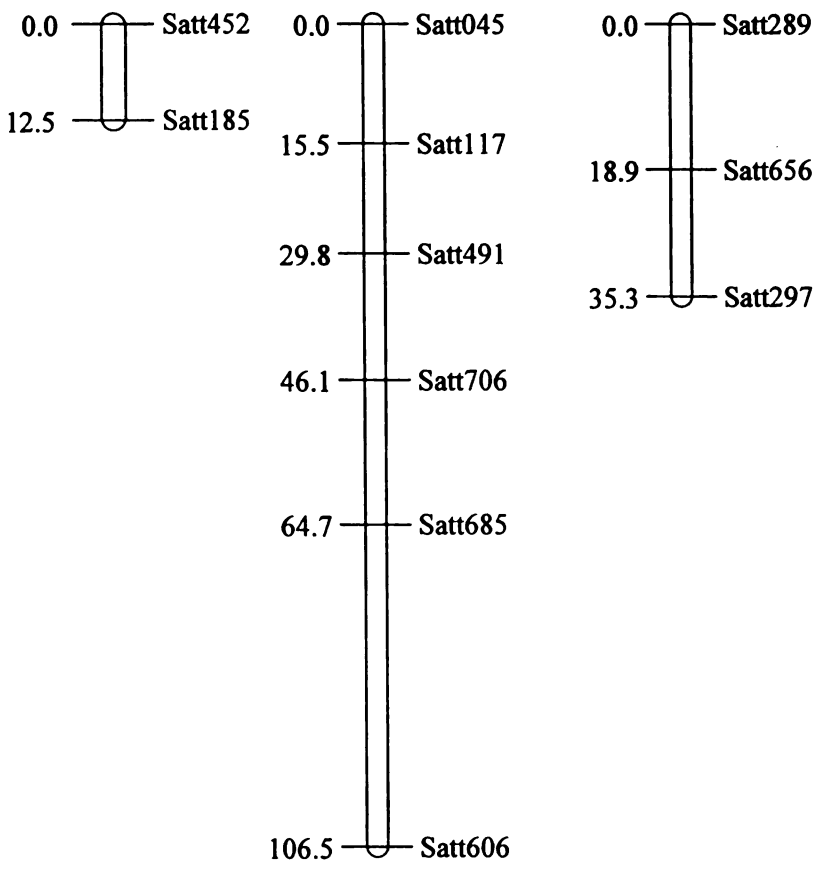

Figure 5.1: Linkage map of 188 F2 lines from cross Titan and PI 567598B constructed using JoinMap 3.0 with a 0D grouping threshold 3.0. The linkage groups were named according to Song et al. (2004) and the map distances between the markers are given in cM (centiMorgans)

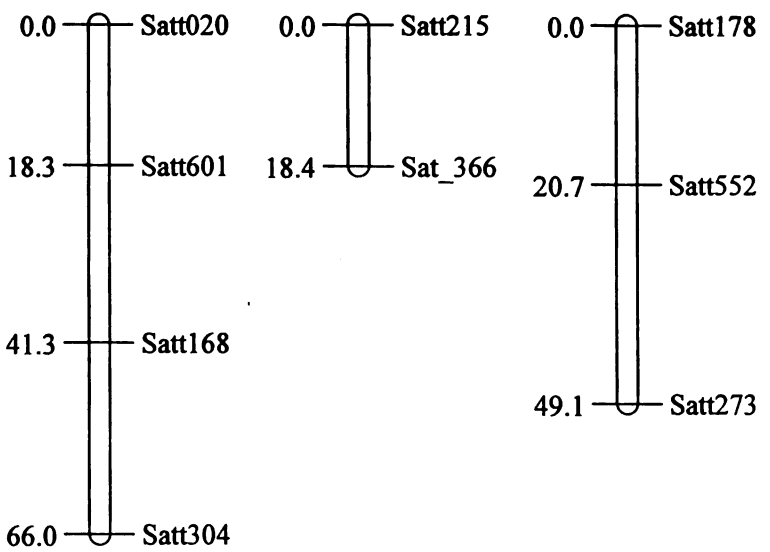
LGK
LGF
LGK
0.0
Satt020
18.3
Satt601
41.3
Satt168
66.0
Satt304
0.0
Satt215
18.4
Sat_366
0.0
Satt178
20.7
Satt552
49.1
Satt273

Fig 5.1 (cont'd)


LGA1
LGJ
LGD1b
0.0
Satt211
27.3
Satt236
51.6
Satt545
0.0
Satt280
24.0
Satt529
0.0
Staga002
20.3
Satt271
43.9
Satg002

Fig 5.1 (cont'd)

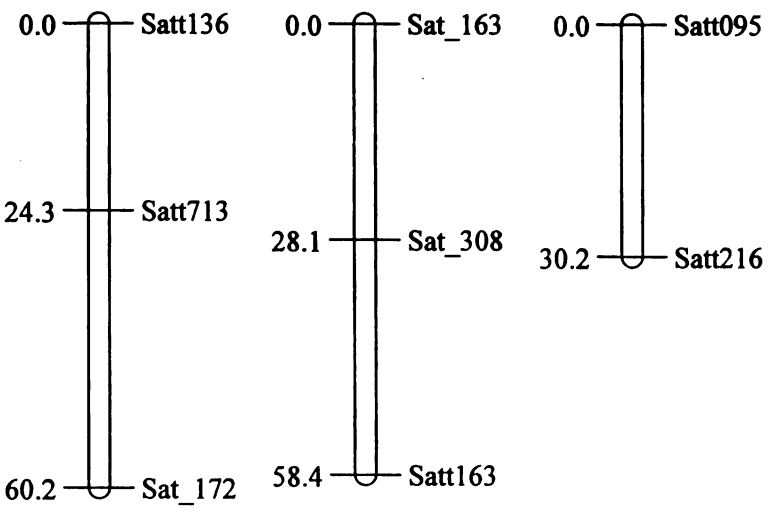
LGC1
LGG
LGD1b
0.0
Satt136
24.3
Satt713
60.2
Sat_172
0.0
Sat_163
28.1
Sat_308
58.4
Satt163
0.0
Satt095
30.2
Satt216

Fig 5.1 (cont'd)

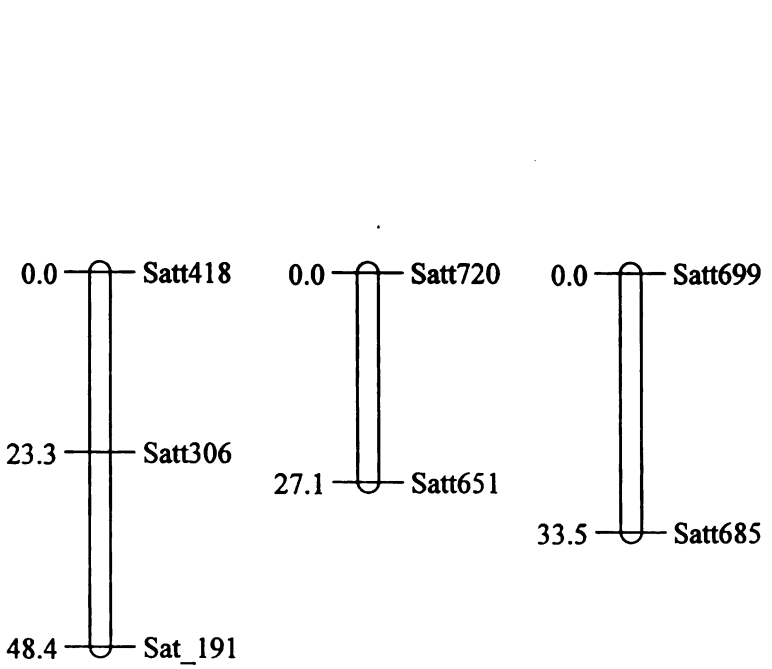
LGL
LGE
LGE
0.0
Satt418
23.3
Satt306
48.4
Sat_191
0.0
Satt720
27.1
Satt651
0.0
Satt699
33.5
Satt685

Fig 5.1 (cont'd)

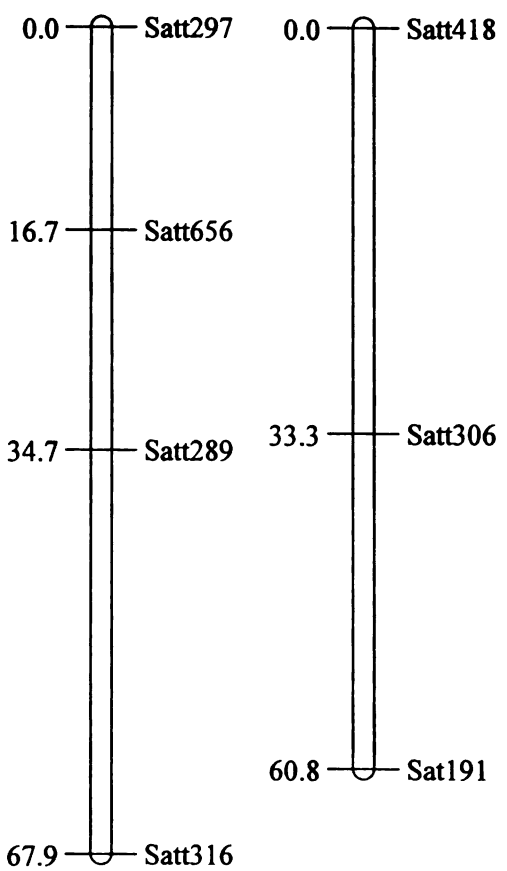
LGF
LGM
0.0
Satt297
16.7
Satt656
34.7
Satt289
67.9
Satt316
0.0
Satt418
33.3
Satt306
60.8
Sat191

Fig 5.1 (cont'd)

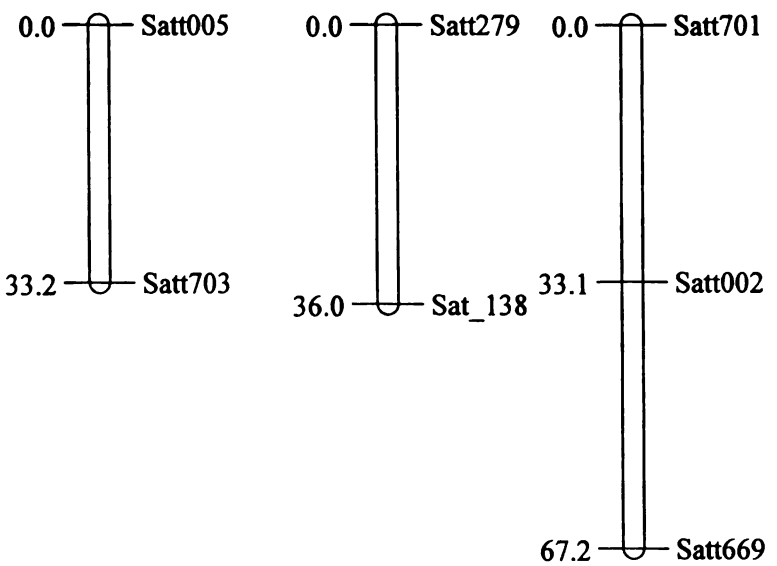
LGD1b
LGH
LGD1b
0.0
Satt005
33.2
Satt703
0.0
Satt279
36.0
Sat_138
0.0
Satt701
33.1
Satt002
67.2
Satt669

Fig 5.1 (cont'd)

Table 5.3: Information about all polymorphic simple sequence repeat (SSR) markers from F_2 population of Titan and PI 567598B.

Marker name	Integrated map (cM)	a : h: b	X^2	Significance level†
Linkage Group **A1**				
Satt211	71.39	39:74:39	0.6	-
Satt236	93.23	25:42:27	1.1	-
Satt545	95.96	39:74:39	0.1	-
Linkage Group **A2**				
Satt589	33.96	58:92:48	4.8	*
Satt177	36.77	34:32:23	9.7	***
Satt187	54.92			-
Satt341	77.7	16:29:36	16.4	****
Sat_138	123.257	25:14:51	21.6	****
Linkage Group **B1**				
Satt665	96.36	36:70:48	3.1	-
Linkage Group **B2**				
Satt168	55.2	50:88:46	0.5	-
Satt304	65.55	47:67:72	18.5	*****
Sat_355	66.235	11:17:16	3.4	-
Satt020	72.13	41:95:50	1	-
Satt066	78.844	25:14:51	57.7	****
Satt070	72.808	40:18:16	35.1	****
Satt063	93.488	22:22:47	38.0	****
Satt474	75.346	25:51:18	1.7	-
Satt601	67.23	18:51:24	1.6	-
Linkage Group **C1**				
Satt661	74.36	34:77:46	1.9	-
Satt136	75.11	48:96:44	0.3	-
Satt361	75.52	22:66:60	21.2	*****
Satt713	88.95	49:98:35	1.9	-
Linkage Group **C2**				
Sat_246	91.81	16:26:14	95.2	*****
Satt376	97.83	50:39:36	20.8	*****
Satt289	112.35	34:59:68	14.4	****
Satt316	127.69	64:56:38	21.9	*****
Linkage Group **D1a**				-
Satt321	50.16	35:79:41	0.1	-
Sat_346	53.671	44:00:00	132	*****
Satt295	55.221	19:39:34	7	**
Satt580	62.367	29:40:25	2.4	-
Satt077	77.49	23:33:38	13.1	****
Satt408	106.69	35:89:46	2	-

† "-" means not significant at 0.05 probability level; *,**, and *** means significant at 0.05, 0.01, and 0.001 probability levels, respectively.

Table 5.3 (Cont'd)

Marker name	Integrated map (cM)	a:h:b	X^2	Significance level†
Linkage Group **D1b**				
Satt095	25.6	54:72:48	5.6	*
Satt701	40.04	26:63:24	1.6	-
Satt005	75.29	36:78:51	3.2	-
Satt005	75.29	36:78:51	3.2	-
Satt350	76.6	35:19:29	25.3	*****
Satt703	98.74	26:49:37	3.9	-
Staga002	126.45	13:38:22	2.3	-
Satt271	137.06	38:72:58	8.2	**
Linkage Group **D2**				
Satt002	47.7	32:63:39	1.2	-
Satt669	67.7	26:47:49	15.1	*****
Satt397	69.296	35:33:16	12.4	****
Satt311	84.62			-
Linkage Group **E**				
Satt720	20.80	63:83:29	13.7	****
Satt651	32.10	55:94:29	8.2	**
Satt606	39.77	17:36:40	16.1	****
Satt699	41.24	44:91:46	0.1	-
Satt602	41.68	37:0:48	87.8	****
Sat_172	42.74	19:66:6	22.2	****
Sat_380	43.29	39:28:21	19.0	****
Satt706	43.36	19:58:39	4.3	-
Satt491	43.64	22:46:25	0.2	-
Satt185	44.76	61:1:29	10.9	***
Satt452	45.10	65:3:25	11.6	***
Satt117	45.78	22:46:26	0.4	-
Satt045	46.65	20:46:28	1.4	-
Satt685	56.69	51:69:52	6.7	**
Satt553	67.92	50:0:39	91.2	****
Linkage Group **F**				
Satt656	22.67	43:83:49	0.9	-
Linkage Group **G**				
Satt163	0.00	31:68:74	29.3	*****
Satt275	2.20	51:91:45	0.5	-
Sat_168	3.90	62:8:23	9.7	***
Sat_163	10.06	16:49:29	3.8	-
Satt356	12.18			-

"-" means not significant at 0.05 probability level; *,**, and *** means significant at 0.05, 0.01, and 0.001 probability levels, respectively

Table 5.3. (Cont'd)

Marker name	Integrated map(cM)	a:h:b	X^2	Significance level†
Linkage Group G				
Sat_315	27.48	15:16:63	89.9	****
Sat_308	43.09	14:56:24		-
Sat_358	45.49	68:0:26	13.2	****
Sat_223	61.64	66:2:26	12.0	****
Linkage Group H				
Satt253	67.17	22:21:50	44.8	*****
Satt279	68.5	137:00:00	41.1	*****
Satt353	84.8	44:79:55	3.6	-
Linkage Group I				
Satt650	63.33	50:106:31	7.2	**
Satt623	95.519			-
Linkage Group J				-
Satt285	25.51	31:44:18	3.9	-
Satt280	38.23	33:87:50	3.5	-
Satt686	40.67	48:80:46	1.2	-
Satt529	41.19	37:98:53	3.1	-
Satt622	42.35	22:46:25	0.2	-
Satt380	43.11	39:28:21	19.0	****
Satt215	44.81	19:43:22	0.3	-
Sat_366	52.10	25:36:30	4.5	-
Linkage Group K				
Satt178	40.80	39:97:52	2	-
Satt552	46.44	43:76:50	2.3	-
Satt628	49.59	70:74:41	16.5	*****
Satt673	50.80	40:104:43	2.5	-
Satg002	51.45	28:103:55	10	***
Satt273	56.62	44:96:43	0.5	-
Linkage Group L				
Sat_191	23.1	30:62:55	12.1	****
Satt418	30.93	35:94:42	2.3	-
Satt313	34.54	46:80:51	1.9	-
Linkage Group M				
Satt636	5	48:90:45	0.1	-
Satt435	38.94	55:93:40	2.4	-
Sat_244	48.85	24:29:69	66.8	*****
Satt323	60.05	25:21:23	10.7	***
Satt306	80.01	34:85:36	1.5	-
Satt250	107.7	45:87:42	0.1	-

"-" means not significant at 0.05 probability level; *,**, and *** means significant at 0.05, 0.01, and 0.001 probability levels, respectively

Table 5.3. (Cont'd)

Marker name	Integrated map(cM)	a:h:b	X^2	Significance level†
Linkage Group N				
Satt675	34.67	24:46:44	11.3	****
Satt660	72.6	54:62:42	9.1	**
Satt255	76.49	48:74:37	2.3	-
Linkage Group Unknown				
Sat_178		37:36:21	10.6	***
Satt024		69:41:49	42.3	*****
Satt059		127:19:39	200.5	*****
Satt098		50:94:43	0.5	-
Satt109		42:84:62	6.4	**
Satt171		50:92:43	0.5	-
Satt216		34:40:20	6.3	*
Satt297		45:95:47	21.9	*****

"-"means not significant at 0.05 probability level; *,**, and *** means significant at 0.05, 0.01, and 0.001 probability levels, respectively